PROCEEDINGS

Volume 64

WESTERN SECTION
AMERICAN SOCIETY
OF ANIMAL SCIENCE

Bozeman, MT

June 18–20, 2013

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MT -A. J. Roberts, Past President (13)
CO-J. C. Whittier, ASAS Board Director (13)
MN-C. K. Larson, Industry Director (15)
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CO-S. L. Archibeque (13)
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WSASAS Business Meeting

Thursday, June 19, 2012 – 7:30 to 9:00 a.m. MDT

Minutes

2011/12 WSASAS Officers:
A. Roberts, President; G. Duff, President Elect; B. Taylor, Secretary/Treasurer; C. Larson, Industry Representative; R. Cockrum and L. Camacho, Graduate Student Representatives; J. Whittier, ASAS-Western Board Director.

1. Call to Order: A. Roberts called the meeting to order at 07:30 a.m.
2. Approval of the Agenda (see appendix 1): Item 4.2: Replace J. Sartin with M. Benson; Item 4.4: Replace D. Crews with A. Roberts; Item 4.5: Replace D. Rule with R. Endecott; and Item 5.2: delete and replaced with "Comments on future publication of WSASAS Proceedings." Modified agenda was approved by general consent.
3. Approval of the Minutes (see WSASAS Proceedings Vol. 63): M. Salisbury moved and H. Neibergs second a motion to approve the 2011 Business Meeting minutes. A. Roberts called for comments/questions; none were made. Motion passed unanimously.
4. Reports
   4.1. Financial: J. Hemmelgarn presented the report (see appendix 2). A. Roberts called for comments/questions; none were made. Report was accepted by general consent.
   4.2. ASAS President: M. Benson presented the report. A. Roberts called for comments/questions; none were made.
   4.3. WSASAS President: A. Roberts presented the report. A. Roberts called for comments/questions; none were made.
   4.4. WSASAS Committees
   - Advisory and Coordinating: M. Salisbury presented the report (see appendix 3). A. Roberts called for comments/questions; none were made.
   - Necrology: A. Roberts reported that 2 former members of the WSASAS, Tom Dunn and Dave Morris, had passed away this past year. A. Roberts called for additional names for the Necrology report; none were made. A. Roberts called for a monument of silence and remembrance for Tom Dunn and Dave Morris.
   - Nominating: G. Duff reported the results of the 2012 WSASAS officer elections, which were:
     - Glenn Duff – President
     - J. Bret Taylor – President Elect
     - Jim Berardinelli – Secretary/Treasurer
     - Connie Larson – Industry Representative
     - Kelsey Quinn – Graduate Student Representative. A total of 5 graduate students were nominated for the position.

A. Roberts called for comments/questions; none were made.

- Beef Symposium: G. Duff reported that the Beef Symposia were well attended and successful. A. Roberts called for comments/questions; none were made.
- Awards: G. Duff presented the report (see appendix 4a). C. Larson presented the Applied Animal Science Award supplemental report (see appendix 4b). A. Roberts called for comments/questions; none were made.
- Graduate Student Paper Competition: H. Neibergs presented the report (see appendix 5). A. Roberts called for comments/questions. B. Taylor stated that several

graduate students were not allowed to compete because their membership was expired; advisors should remind graduate students that ASAS/WSASAS membership must be renewed each year.

4.5. Academic Quadrathlon: R. Endecott presented the report (see appendix 6). A. Roberts called for comments/questions. Comments were made about moving the AQ back to spring. However, it was pointed out that WSASAS had recently visited this issue, and WSASAS had voted to hold the AQ joint with WSASAS. Other comments were made about hosting an undergraduate poster/oral competition in conjunction with the AQ and WSASAS annual meeting.

4.6. Graduate Student Representative: R. Cockrum reported that the Young Scholars Award proposal was completed and ready for action and comment from the membership. Further report on the award was carried forward to New Business-Item 5.1.

5. New Business

5.1. Young Scholar Award: B. Taylor moved and J. Whittier a motion to incorporate the Young Scientist Award proposal as presented by R. Cockrum, L. Camacho, and C. Larson (see appendix 7). A. Roberts called for discussion. R. Cockrum stated that if the motion passes, comments were welcomed on how to best launch the program for 2012/13. A. Roberts suggested that a web-based venue should be made available for the membership to provide comments. J. Sprinkle and C. Larson suggested that the Young Scholar Award should not be considered an award. Further comments were made that the name should be changed to Young Scholar Recognition. No other discussion was made. Vote was called and motion passed unanimously.

5.2. Comments on future publication of the WSASAS Proceedings:

- B. Taylor and M. Wulster-Radcliffe presented the current situation with the WSASAS Proceedings. Briefly, FASS will no longer produce the WSASAS Proceedings. For the 2012 Proceedings, ASAS outsourced publication services for the 2012 WSASAS proceedings at 1/3 the cost normally charged by FASS. ASAS-JAS has requested the WSASAS consider: (1) using Manuscript Central as the submission venue for future proceedings, (2) changing the proceedings to a peer-reviewed publication, (3) changing the style and format to match JAS, (4) establishing a memorandum-of-understanding with ASAS-JAS on how the proceedings is to me managed in the future. A. Roberts called for comments/questions, which were voiced by D. Hallford, J. Sprinkle, S. Ivey, J. Whittier, T. Ross, A. Roberts, C. Schauer, and G. Duff. Questions/Comments were: (1) Will publishing data in a "peer-reviewed" WSASAS Proceedings preclude the data from being published in a peer-reviewed journal as original research? (2) Can proceedings submissions be rejected? If rejected, how will appeals be handled? (3) Will editors for the proceedings be selected within the Western Section membership? (4) Will the volume number be consecutive with pervious proceedings? (5) Will the "look" be altered? (6) Will submission deadlines be earlier in the year? (7) Will ASAS, JAS, or WSASAS received funds from proceeding sales? (8) How will "proceeding submitters" that have no intention of presenting at the WSASAS meeting be identified and culled from the final proceedings publication? (9) How will proceedings papers for the Graduate Student Paper Competition be handled?

- A. Roberts stated that an e-based comment and question venue will be establish to receive input from the WSASAS membership before the WSASAS Executive Committee makes a final decision.
- A. Roberts established a committee to work with ASAS-JAS in finalizing the publication processes of future WSASAS Proceedings. Volunteers were: D. Hallford, J. Whittier, B. Taylor, and C. Schauer.

6. Transfer of the Gavel: A. Roberts transferred to gavel to President-elect, G. Duff. G. Duff presented A. Roberts with service recognition plaque.
7. Adjourn – G. Duff adjourned the meeting at 8:56 a.m.

Appendices

Appendix 1

WSASAS Business Meeting

Thursday, June 19, 2012 – 7:30 to 9:00 a.m. MDT

Sheraton, Phoenix – South Mountain Room

AGENDA

1. Call to Order	A. Roberts
2. Approval of the Agenda	A. Roberts
3. Approval of the Minutes	A. Roberts
4. Reports	
4.1. Financial	B. Taylor, J. Friedrich
4.2. ASAS President	J. Sartin
4.3. WSASAS President	A. Roberts
4.4. WSASAS Committees	
• Advisory and Coordinating	M. Salisbury
• Necrology	D. Crews
• Nominating	D. Crews
• Symposiums	
o Beef Symposium, Systems	G. Horn
o Beef Symposium, Arid Environments	R. Funston
o Beef Symposium, Stress	G. Duff
• Awards	G. Duff
• Graduate Student Paper Competition	
o 2012 competition	H. Neibergs
o Membership, abstracts, and proceedings	M. Wulster-Radcliffe, B. Taylor
4.5. Academic Quadrathlon	D. Rule, M. Wulster-Radcliffe
4.6. Graduate Student Representative	R. Cockrum, L. Camacho
5. New Business	
5.1. Young Scholar Award	R. Cockrum, L. Camacho, C. Larson
5.2. Policy: “zero nominations” for awards	M. Salisbury
6. Transfer of the Gavel	G. Duff
• Adjourn	

Appendix 2

American Society of Animal Science					
Financial Report-Western Section					
For the Five Months Ending May 31, 2012					
	Actual @ 05/31/12	*Actual @ 05/31/11*	*Actual @ 05/31/10*	*Actual @ 05/31/09*	*Actual @ 12/31/11*
Balance as of January 1	***$51,549.34***	***$58,404.68***	***$55,119.98***	***$55,896.83***	***$58,404.68***
Revenue and Support					
Dues-ASAS					1,316.00
Registrations		8,230.00			14,475.00
Ticketed Events	1,600.00	2,441.00	1,290.00		4,139.00
Donations-Awards	1,050.00	700.00	2,900.00	700.00	1,250.00
Donations-Symposium		2,830.00			4,760.00
Symposium Support-ASAS					3,000.00
Proceedings	9,150.00	8,305.00	2,850.00	1,495.00	9,090.00
Investment Earnings Gain(Loss)	550.97	2,566.70	(499.48)	872.50	(872.53)
Total Revenue and Support	**12,350.97**	**25,072.70**	**6,540.52**	**3,067.50**	**37,157.47**
Expenses					
Programs/ Registration					221.22
Awards/Plaques					6,403.00
Quadrathalon				3,600.00	4,389.95
Convention Center					10,370.61
Travel-Staff				1,158.43	4,687.89
Marketing	864.99	3,143.01			3,272.55
Proceedings					1,406.63
Postage, Shipping & Supplies	17.09	8.70		7.88	638.15
Writer's Workshop				443.19	
Miscellaneous					1,363.80
Insurance	139.05				111.24
Telephone				96.86	
General Printing					345.48
Staff Support	2,297.08	4,017.39	2,984.94	4,007.80	10,802.29
Total Expenses	**3,318.21**	**7,169.10**	**2,984.94**	**9,314.16**	**44,012.81**
Net Revenue over Expense	**9,032.76**	**17,903.60**	**3,555.58**	**(6,246.66)**	**(6,855.34)**
Balance as of December 31	***$60,582.10***	***$76,308.28***	***$58,675.56***	***$49,650.17***	***$51,549.34***

Appendix 3

2012 Western Section ASAS Advising and Coordinating Committee Report
Mike Salisbury, Chair
Angelo State University, San Angelo, TX

Assignment: On May 31, 2012, the Advising and Coordinating Committee was assigned the task to draft a policy on Award Nomination Deadlines.

Background: In past years, there are often no or no qualified nominations for the awards presented at the WSASA annual meeting. Often times the deadlines had been extended to allow more time for making nominations. However, due to the fact that a policy had not been established on how the deadline should be administered annually the committee was requested to meet and draft a recommendation for establishing award nomination deadlines.

The committee met via email May 31, 2012 – July 3, 2012 to discuss options, draft a policy and approve a draft policy for submission to the Executive Board.

Members (Participated in Discussion):

TX–M. W. Salisbury (Participated)
AK–M. P. Shipka (Participated)
CO–J. E. Bruemmer
NM–D. M. Hallford (Participated)
ID–J. B. Glaze (Participated)
WY–K. M. Cammack
WY–R. R. Cockrum
ND–L. E. Camacho
MT–R. C. Waterman (Participated)
MT–J. G. Berardinelli (Participated)
NM–E. J. Scholljegerdes (Participated)

Following much discussion, the committee was split 4 to 3 on a policy. Since the vote was so closely divided, two policies were drafted for the Executive Board to consider. Policy 1 received 4 votes and Policy 2 received 3 votes.

I. Award Nomination Policy – No Deadline Extension

The WSASAS Secretary/Treasurer will solicit nominations for society awards from WSASAS members via mailings, e-mail notifications and/or website postings. Award nominations will cease on the deadline set by the WSASAS Secretary Treasurer. If no nominations for a particular award are submitted, the award will not be given that year. In the event that nominations (one or more) are submitted before the deadline, the WSASAS Awards Committee will evaluate the submissions and determine if a qualified individual is among the nominees. If the committee determines there are no qualified nominees for a particular award, the award will not be given that year.

II. **Award Nomination Policy – Deadline Extension**

The WSASAS Secretary/Treasurer will solicit nominations for society awards from WSASAS members via mailings, e-mail notifications and/or website postings. Award nominations will cease on the deadline set by the WSASAS Secretary Treasurer. If no or very few nominations for a particular award are submitted by the original deadline, a two (2) week deadline extension will be imposed. Following the two (2) week deadline extension, if no nominations for a particular award have been received, the award will not be given that year. In the event that nominations (one or more) are submitted (before original or extended deadline), the WSASAS Awards Committee will evaluate the submissions and determine if a qualified individual is among the nominees. If the committee determines there are no qualified nominees for a particular award, the award will not be given that year.

Appendix 4a

2012 Western Section ASAS Awards Committee Report
Glenn Duff, Chair
Montana State University, Bozeman

Committee Membership
G. C. Duff, President-Elect (12, Montana State University, Bozeman)
A. L. Van Eenennaam, (12, Univeristy of California-Davis)
A. Ahmadzadeh (12, University of Idaho)
S. Archibeque (13 Colorado State University)
S. I. Paisley (13, University of Wyoming)
D. C. Rule (13, University of Wyoming)

2011 Recipients

Distinguished Service Award

Recipient: Dr. Doug Hixon, University of Wyoming
Sponsor: DSM Nutritional Products, Inc.
c/o Scot Williams and Yvonne Towns
45 Water View Blvd.
Parsippany, NJ 07054-1298
Nominators: Dr. David R. Ames, Colorado State University

Distinguished Teaching Award

Recipient: Dr. James G. Berardinelli, Montana State University
Sponsor: Elanco Animal Health
c/o Dr. Todd Armstrong
2001 W. Main Street
PO Box 708
Greenfield, IN 46140-2714
Nominators: Dr. Glenn C. Duff, Montana State University

Extension Award

Recipient: Dr. Rachel Endecott, Montana State University
Sponsor: Western Section ASAS
Nominator: Dr. Glenn Duff, Montana State University

Young Scientist Award

Recipient: Dr. Shanna L. Ivey, New Mexico State University
Sponsor: Western Section ASAS
Nominator: Dr. Tim T. Ross, New Mexico State University

Drs. Andy Roberts and Glenn Duff presented Distinguished Teaching, Extension and Young Animal Scientist Awards and Dr. David Ames and Andy Roberts presented the Distinguished Service Award at the banquet on July 18, 2012. Glenn Duff thanked all who submitted nominations and encouraged nominators to get to work early and nominate our deserving colleagues in 2013.

Appendix 4b

2012 Applied Animal Science Award
Connie Larson

16 papers submitted
- University of Wyoming: 2
- Colorado State University: 2
- South Dakota State University: 2
- North Dakota State University: 2
- New Mexico State University: 2
- Montana State University: 3
- Oklahoma State University: 1
- University of Nebraska-Lincoln: 2

Committee Members: 18 total (14 industry sponsors and 4 academic participants)

Tim Bodine	PerFormix Nutrition
Mark Branine	Pfizer
Dan Dhuyvetter	Ridley Block
Kristy Dorton	Diamond V
Allison Grove	AG Research, LLC
Kim Hagar	CHS
Jeff Heldt	LOL Purina
Jim Killen	Strauss Feeds
Connie Larson	Zinpro
Jeremy Martin	Great Plains Consulting
Trey Paterson	Padlock Ranches
Sonda Sibole	IMI Global
Kelcey Swyers	Ranchway Feeds
Gary Tibbetts	Zinpro
Court Campbell	
Mike Hubbert	NMSU
Mark Petersen	Ft. Keogh
Burke Theikert	

Placing:

1st Place: $500.00: Metabolizable protein supply alters pregnancy and subsequent retention rate during heifer development while grazing dormant winter forage. J. T. Mulliniks[†]. D. E. Hawkins[§], K. K. Kane†, S. H. Cox†, L. A. Torell†, E. J. Scholljegerdes[†], and M. K. Petersen; [†]New Mexico State University, Las Cruces, NM, 88003; [§]West Texas A&M University, Canyon, TX, 79016; [¶]USDA-ARS Fort Keogh Livestock and Range Research Laboratory, Miles City, MT 59301

2nd Place: $300: Effects of post-AI nutrition on growth performance and fertility of yearling beef heifers. R. P. Arias[1], P. J. Gunn[2], R. P. Lemenager[2], and S. L. Lake[1];[1]Animal Science Department, University of Wyoming, Laramie, WY; [2]Department of Animal Sciences, Purdue University, West Lafayette, IN

3rd Place: $200: Heifers with low antral follicle counts have low birth weights and produce progeny with low birth weights. A. F. Summers[1], R. A. Cushman[2], and A. S. Cupp[1]. [1]University of Nebraska- Lincoln, Lincoln, NE; [2]USDA-ARS U.S. Meat Animal Research Center, Clay Center, NE

Appendix 5

Graduate Student Competition Committee Report – 2012
Holly Neibergs, Washington State University

Committee members
Holly Neibergs, Washington State University, Chair; Shanna Ivey, New Mexico State University; Rachel Endecott, Montana State University; Kraig Peel, Colorado State University; Chad Mueller, Oregon State University; Eric Scholljegerdes, New Mexico State University; Jason Ahola, Colorado State University; Scott Lake, University of Wyoming

The committee nominates Chad Mueller (Oregon State University) as committee chair for 2013. Holly Neibergs, Shanna Ivey and Rachel Endecott have completed their three year terms. The committee recommends Ryan Ashley (New Mexico State University), Chris Schauer (North Dakota State University), and Michelle Mousel (USDA, ARS Dubois) as replacement members.

Competition
The GSC Committee received 28 abstracts; 6 abstracts were not considered for the competition due to failure to meet proceedings deadlines or scheduling conflicts. Twenty-six of the abstracts were presented during the session as two abstracts (that were not under consideration for the competition) were not presented. Therefore, 22 abstracts were considered for competition:

J.T. Mulliniks, New Mexico State University; M.G. Dib, Colorado State University; M.L. Van Emon, North Dakota State University; E.A. Bailey, Kansas State University; K.P. Sharon, Montana State University; J.M. Sieg, Utah State University; M.V. Sis, Colorado State University; P.E. Repenning, Colorado State University; K.E. Quinn, New Mexico State University; E.E. Nix, Montana State University; L.E. Camacho, North Dakota State University; P.L. Steichen, North Dakota State University; K.L. Weber, University of California at Davis; B.J. Bigler, Colorado State University; R.R. Cockrum, University of Wyoming; C.G. Jackson, North Dakota State University; P.L. Black, New Mexico State University; M.K. Beckman, New Mexico State University; C.A. Roberts, South Dakota State University; D.L. Ragen, Montana State University; N.L. Hojer, South Dakota State University; Q.P. Larson, North Dakota State University;

Considerations
Conflicts of interest were identified; those with conflicts of interest did not participate in judging and discussion of the respective paper/presentation.

Results
The results of the GSC were tabulated and awards were presented at the WSASAS awards banquet. Individual awards were:

First Place: M.K. Beckman, New Mexico State University
Digestibility of algal biofuel co-product in a forage diet. M.K. Beckman, L.N. Tracey, N. Miller, K. Norman, K. Marchetti, E.J. Scholljegerdes, S.A. Soto-Navarro, C.L. Loest, and S.L. Lodge-Ivey.

Second Place: K.E. Quinn, New Mexico State University
Fetal and maternal induction of angiogenic factors during early pregnancy. K.E. Quinn, J.D. Lindsey, S.M. Stanbrough, A.K. Ashley, and R.L. Ashley.

Third Place Tie: L.E. Camacho, North Dakota State University and C.A. Roberts, South Dakota State University
Maternal diet restriction in beef cows alters fetal cardiovascular hemodynamics and fetal and placental development during early pregnancy. L.E. Camacho, C.O. Lemley, K.C. Swanson, and K.A. Vonnahme.

Institutional Award. The institutional award for the highest average score with 2 or more contestants was presented to the **New Mexico State University** by Connie Larson from Zinpro Corporation. WSASAS expresses its gratitude to Zinpro and Connie Larson for their continued support of the Graduate Student Competition and the Institutional Award.

Appendix 6

WESTERN SECTION ASAS ACADEMIC QUADRATHLON REPORT

Rachel Endecott – Montana State University

The Western Section Academic Quadrathlon was held in conjunction with the seminal National ASAS Quadrathlon. The following universities participated in this year's event: New Mexico State University; Colorado State University; University of Wyoming; Utah State University; Oregon State University; and Montana State University.

The contest began on the University of Arizona campus in Tucson with the Written Exam followed by the Lab Practicum events. The University of Arizona provided a first class event with equally fine accommodations; many thanks to the University of Arizona! The teams returned to Phoenix to complete the remaining two events, which were held Saturday the 14th. The results of the contest are shown in the table below. Congratulations to Colorado State University for winning the overall and moving onto the National AQ event to represent the Western Section. Colorado State University will have their scores from the Written Exam, Lab Practicum, and Oral Presentations events entered along with those respective scores from the winners of the other three regions: Ohio State University, Penn State University, and Texas A&M University. The rankings within the National event will be combined with the results of the National ASAS quiz bowl to determine the National ASAS AQ champion.

SCHOOL	QUIZ BOWL	WRITTEN	LAB PRACTICUM	ORAL PRESENTATION	OVERALL
UTAH ST.	1	4	4	5	3.5
OREGON ST.	2	3	5	4	3.5
MONTANA ST.	3	5	2	6	5
WYOMING	4	2	3	2	2
NEW MEXICO ST.	5.5	6	6	3	6
COLORADO ST.	5.5	1	1	1	1

The Western Section AQ advisors met to discuss several issues. The first involved awards. The advisors agreed to develop a traveling trophy instead of plaques; we will begin this with the 2013 contest. The second issue discussed was moving the WS AQ back to the Spring Semester because of the new hardship that the winning team will have traveling to both the June WS ASAS meetings and then again in July to the National ASAS for the National AQ. The hardship is barely doable with the June meeting, and we cannot expect the students, especially those who graduate during the spring semester, to leave jobs twice for the contest. The advisors voted unanimously to make this move, and it will be in the best interest of the student participants if we pursue this starting in 2013. The Western Section ASAS board will need to approve this move; we encourage the board not to delay the decision so that we can plan for next year's event. Pending WS ASAS board approval, we have tentatively scheduled the 2013 WS AQ for the second weekend in April in Bozeman, Montana.

Dan Rule, WS AQ Chairman

Rachel Endecott: rachel.endecott@montana.edu

Appendix 7

Western Section of ASAS
Announcement of the Animal Science Young Scholars Award

Graduate Student Directors: Rebecca Cockrum and Leticia Camacho

During the 2012 Business meeting of Western Section American Society of Animal Science (WSASAS), members of the WSASAS will be asked to vote on whether to establish a new separate session for the Western Section Animal Science annual meeting. Title of this proposed session is "Young Scholars Award".

Proposal:
The specific aims of this program are to:
1) Acknowledge the research accomplishments of current and/or recent Ph.D. and M.S. students in the Western Section of ASAS, and
2) Generate more member participation through providing a platform that showcases exceptional and contemporary research of future scientists.

Graduate students interested in being considered for the Animal Science Young Scholars Award **must be current members of WSASAS** and must submit a Western Proceedings Paper. Major Professors or individuals closely familiar with the candidate's research and academic achievements can nominate qualified students. From the initial pool of applicants, a Young Scholars Award Committee will identify four Ph.D. and four M.S. students for further consideration. Student selected from the initial pool will then be invited to submit 2 additional letters of recommendation (one letter from the department chair), a 2-page essay discussing their future goals, and an abstract. The Young Scholars Award Committee will then select the top two Ph.D. and top two M.S. students for the Young Scholars Award. Awardees will be required to present a 30-minute presentation during the annual WSASAS meeting on their thesis or dissertation research allowing for a detailed discussion of innovative and relevant research. A $350.00 monetary award, sponsored by Zinpro and a plaque will be presented to each awardee during the WSASAS awards banquet. The Young Scholars Award proposed is to acknowledge the research accomplishments of Ph.D. and M.S. students in Animal Science through an oral presentation format; the scholarship is not proposed as a graduate student competition.

Overview and timeline:

1. Students in their final year or within 12 months of having completed their Masters or Doctorate program are eligible to be considered for the Animal Science Young Scholars Award. **Candidates must be current members of WSASAS.**

2. A call for nominations will be issued in October and nominations will be due by December. Major professors or individuals closely involved with the nominee's research and academic achievements can nominate qualified students. To nominate a student, a one-page letter discussing the student's research and academic achievements and the student's curriculum vitae will be required.

3. Once all nominations have been received, a Young Scholars Awards Committee will review the letters of referral and select the top four Ph.D. students and the top four M.S. students to be further considered for the Young Scholars Award.

4. The top eight selected students will be contacted via e-mail by a committee representative to request 2 additional letters of recommendation (with one letter from the department chair), a 2-page essay discussing their future research goals and potential impacts of future research, and an abstract. Supplemental material will be due back to the Young Scholars Awards Committee by January. From these candidates, two MS and two PhD students will be selected to receive the Award.

5. Final decisions on the Young Scholars Award will be made by Mid- to late January.

6. A member of the Young Scholars Award Committee will contact the selected Young Scholars via phone. Awardees will present research following the WSASAS beef symposium. A 30-minute presentation will be given by each awardee discussing their thesis or dissertation research with time allotted during the 30 minutes for questions.

7. All Young Scholars awardees will be required to submit an abstract and proceedings paper in accordance with the National ASAS and WSASAS schedule.

8. Proceedings papers will follow the guidelines as published by WSASAS and there will be no page fee for the awardees.

9. Students selected for this recognition and who present their research at the joint ASAS and WSASAS meeting will receive a plaque, complimentary meeting registration, and a $350.00 monetary award.

10. Personal statements, recommendation letters, and abstracts should be sent electronically to the Young Scholars Award Committee by early January.

For more information please contact:
Leticia (Ely) Camacho – 2012 Senior Graduate Student Representative
Graduate Research Assistant/Animal Sciences
North Dakota State University
Office 701.231.7630
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Budget:

Item	**Cost**
Costs (2 M.S. and 2 Ph. D. students)	
Monetary award (4 students @ $350.00 each)	$1400.00
Plaques ($50.00 each)	$200.00
Meeting registration ($100.00 each)	$400.00
Proceedings paper ($25.00/page)	$600.00
Revenues	
Zinpro sponsorship	-$2000.00
WSASAS (proceedings paper)	-$600.00
Total	$0.00

*****Western Section Graduate Student Event, Sponsored by Western ASAS

WERA-39-WSASAS Sheep Symposium

Integrating Advanced Concepts into Traditional Practices

June 19, 2013

Museum of the Rockies

Montana State University

Bozeman, Montana

Keynote Speaker: Rodney Kott

Professor and Sheep Extension Specialist, *Montana State University*

Impact of Research on the Sheep Industry

Dr. Rodney Kott received his BS and MS from Texas A&M University and PhD from New Mexico State University in 1980. After receiving his PhD, Dr. Kott became the Extension Sheep Specialist at Montana State University where he continues to serve the Montana Sheep Industry. Dr. Kott has provided leadership to Montana sheep extension programs for more than 30 years and more recently included activities of the Montana Wool Laboratory. Through the years, Dr. Kott has been involved in National efforts such as Targeted Grazing, the National Sheep Improvement Program (NSIP), and the Certified Wool Classing Program. In Montana, Dr. Kott has been involved in the utilization of sheep to manage invasive plants (targeted grazing), the restructuring of the wool pool marketing system, and the use of genetic records at the Miles City Ram Sale. Research emphasis includes winter supplementation, lamb survival, objective measurement of wool and more recently the development of genetic records and selection indexes in sheep, and residual feed intake. Dr. Kott received the Extension Award from the American Society of Animal Science in 1996; Hall of Fame from the US Targhee Association in 2005; Outstanding Extension Worker from MSU Extension in 2007, Agency Weed Fighter from the Montana Weed Control Association in 2008; and the Flock Tender Award from the American Sheep Industry in 2009. Dr. Kott continues to work closely with the Montana Wool Growers Association and the national American Sheep Industry Association.

David Anderson

Professor and Extension Economist, *Texas A&M AgriLife Extension Service*

The U.S. Lamb Market

David Anderson is a Professor and Extension Economist – Livestock Economist -- with the Texas A&M AgriLife Extension Service. After finishing a PhD at Texas A&M University in 1994, he was a livestock market analyst at the Livestock Marketing Information Center in Denver, Colorado. He returned to Texas A&M in 1996. His work involves the analysis of livestock and dairy market economics and policy. He is originally from Coolidge, Arizona where his father is a cotton farmer.

Dr. David Anderson is a Professor and Extension Economist in the Department of Agricultural Economics at Texas A&M University. His research and extension education activities are in livestock, and food products marketing and agricultural policy, focusing on relevant issues for Texas producers. He is the Texas AgriLife Extension Livestock and Food Products Marketing economist.

Dr. Anderson's program has focused research on the impact of alternative farm programs on the livestock, dairy and crop sectors of agriculture. Dr. Anderson has done extension research on wool, mohair, and dairy policy. Recent extension programs have focused on market outlook, animal identification systems, and country of origin labeling. He currently teaches Ag. Econ. 614 which is an applied policy analysis course, primarily for M.S. students.

Dr. Anderson received two degrees in agricultural economics at the University of Arizona and earned a Ph.D. in agricultural economics at Texas A&M University. Prior to returning to Texas A&M in 1996, he worked for the Colorado Agricultural Extension Service, Colorado State University, in the Livestock Marketing Information Center for two years. He has received awards for Professional Excellence from the American Agricultural Economics Association, the Outstanding Extension Program Award from the Western and Southern Agricultural Economics Associations, and the TAMU Deputy Chancellor's Distinguished Performance Team Award for Research and Extension.

Rebecca Cockrum

Postdoctoral Fellow, Animal Science, *Colorado State University*

Applying Genomic Selection Technology to the Sheep Industry

After receiving her B.S. degree in Animal Science at Arkansas State University in 2004, Dr. Cockrum moved to St. Louis, MO to gain experience in industry as a scientific recruiter. Dr. Cockrum was then accepted into the Reproductive Biology program in the Department of Animal Science at the University of Wyoming where she received her M.S. degree in 2009 and Ph.D. in 2012 under the direction of Dr. Kristi Cammack. Dr. Cockrum's thesis research focused on differential gene expression in liver in response to toxins in the diet of sheep. Dr. Cockrum then collaborated with AgResearch in New Zealand to identify ovine markers associated with feed efficiency for her dissertation research. During her 5-year graduate career, Dr. Cockrum presented research at 17 scientific meetings and was an invited speaker at AgResearch, New Zealand, National Lamb Feeders Association, USDA-Meat Animal Research Center, and the University of Wyoming. Dr. Cockrum is the primary author of two peer-reviewed publications, contributing author of five peer-reviewed publications, and has three manuscripts in-progress. A few highlighted achievements include: the first WSASAS graduate student director, ASAS graduate student representative for the Small Ruminant committee, President of the University of Wyoming Animal Science Graduate Student Association, Gamma Sigma Delta Outstanding Ph.D. Student of the Year, and received $24,990 from Western SARE grant program to conduct a portion of her dissertation research. Dr. Cockrum is currently a Postdoctoral Fellow at Colorado State University under the direction of Dr. Milt Thomas. Her current research focuses on characterizing gene regulatory networks involved in hypoxic-induced pulmonary hypertension and bovine respiratory disease in cattle.

Thomas Craig

Professor Veterinary Parasitologist, *Texas A&M*

Sheep Parasites: Problems, Resistance, New Products and Practices for Parasite Control

Thomas M. Craig earned his DVM from Colorado State University, and PhD from Texas A&M University. He served as a military veterinarian in Denmark, and Slovenia, then was in mixed veterinary practice for 7 years in Colorado, New Mexico and New Zealand; where grazing animals were a significant part of practice activities. Presently he is a faculty member in the College of Veterinary Medicine Texas A&M University where he does research, diagnosing and teaching veterinary parasitology as a professor in the Department of Veterinary Pathobiology (worms, germs and things too gross to mention). His teaching activities are to 2nd and 4th year veterinary students; covering aspects basic and clinical parasitology, graduate students, continuing education for veterinarians, and livestock owners and introductory parasitology to high school and pre-high school students: Vet camps, 4-H, Pony Clubs. Over the years he has engaged in research primarily concerned with the epidemiology and control of internal parasites of grazing animals, the diagnosis, epidemiology and treatment of arthropod borne disease and the evaluation of anthelmintics in laboratory and field conditions. He also serves as the director of the parasitology diagnostic laboratory; servicing the veterinary teaching hospital, veterinary practitioners, the Texas Veterinary Medical Diagnostic Laboratory and animal owners. He consults with veterinary practitioners, animal owners and pharmaceutical companies. Over the years he helped raise two wonderful children, cattle, sheep, and horses for fun and occasional profit.

Peter Orwick

Executive Director, *American Sheep Industry*

ASI Initiatives for Industry Growth

As ASI's executive director since 1997, Peter Orwick has spearheaded major initiatives including the first successful Trade Action on imported lamb meat from Australasia and the wool marketing loan, which combined, resulted in more than $250 million in federal funding for the U.S. sheep industry. Established the national scrapie disease eradication program, first industry wide promotion checkoff for lamb with packers, feeders and producers contributing, created a livestock risk insurance for lamb, and funded a multi-million dollar international marketing program for wool that doubled American wool exports. Orwick led creation of Mandatory Price Reporting, national sheep industry improvement center, and the Sheep Venture Company under ASI with multi millions of dollars in assets including wool superwash textile equipment, lamb insurance and military product development research. Prior to his executive director position, Orwick served as ASI's director of government affairs from August 1991 to February 1997, during which he oversaw the association's government relations and natural resources programs.

Before joining the ASI staff, Orwick was appointed director of the South Dakota Department of Agriculture's Division of Conservation by Governor George Mickelson to manage soil and water conversation programs statewide. Orwick initiated the plan that would become the state's official soil and water conservation guidance. Orwick was reared on a sheep and cattle ranch on the rangelands of western South Dakota. His parents, brother and sisters are sheep ranchers with several thousand Rambouillet ewes. He received bachelor of science degrees in animal science and business from South Dakota State University in 1984.

Kim Vonnahme

Associate Professor, Animal Scientist, *North Dakota State University*

Maternal Environment Impacts Fetal and Offspring Outcomes in Sheep

Kimberly Vonnahme grew up on a livestock and grain farm near Breda, Iowa (West-Central Iowa) and is the second oldest of five children. Upon graduation from high school, Kim attended Iowa State University majoring in Animal Science. Her interest in reproductive biology was sparked, and after graduation in 1996, pursued her Master of Science degree at Oklahoma State University under the guidance of Dr. Rodney Geisert working on embryo-uterine interaction during early pregnancy in the pig with a thesis entitled "Detection of Glandular Kallikrein and Low Molecular Weight Kininogen in the Porcine Uterus during the Estrous Cycle and Early Pregnancy". She returned to Iowa State University in 1998 to begin her PhD program with Dr. Steve Ford, and moved with Ford to the University of Wyoming in 2000, where she completed her PhD degree in 2003. While her dissertation title was "The Impacts of Placental Size and Vascularity on Litter Size in the Pig", Kim also helped with the early studies in the Center for the Study of Fetal Programming developing a nutritional model using pregnant sheep. In the fall of 2003, Kim moved to North Dakota State University with interests in learning measurements of vascularity in sheep and cow placenta from Dr. Larry Reynolds. In April, 2004, Kim accepted an assistant professor position in the Department of Animal Sciences to teach and conduct research. She became Associate Professor in 2010. Dr. Vonnahme's research programs focuses on the impacts of maternal nutrition on fetal and placental development in sheep and cattle. More specifically, Kim is interested in how the maternal nutrition impacts uteroplacental blood flow, development of the placenta, and nutrient transfer. Kim is married to Michael Kangas and they have 2 children, Katie and Joey.

John Walker

Professor and Resident Director of Research, *Texas A&M AgriLife Research*

Sheep, Black Swans and the Future of Agriculture

Dr. Walker has served as Professor and Research Director at the Texas A&M University Agricultural Research and Extension Center in San Angelo, Texas since 1997. His responsibilities include providing leadership to a multi-disciplinary team of scientists that develop new technologies for increasing the efficiency and sustainability of range livestock and wildlife production. His area of expertise involves foraging ecology, modification of livestock foraging behavior and targeted livestock grazing for landscape enhancement. Dr. Walker developed the first fecal near-infrared spectroscopy calibrations for predicting botanical composition of ruminant diets. He continues to develop near-infrared spectroscopy solutions in support of research in foraging ecology, and animal nutrition. Prior to his current position Dr. Walker was a rangeland scientist for the Agricultural Research Service at the U.S. Sheep Experiment Station in Dubois, Idaho. In this position he conducted research to improve sustainable utilization of rangelands. Studies included combination grazing of sheep and cattle; use of grazing livestock to control noxious weeds; genotypic and phenotypic factors affecting foraging behavior; weed ecology; low input sustainable sheep production systems; and carbon budgets on sagebrush steppe rangelands. Dr. Walker received his B.S. in wildlife science and Ph.D. in rangeland ecology both from Texas A&M and his M.S. in range science from Colorado State University. He has authored or co-authored 60 scientific journal articles and over 100 other professional publications. Dr. Walker serves on numerous professional and civic boards and committees including the Targeted Grazing Committee both for the Society for Range Management and the American Sheep Industry Association.

Travis Whitney

Associate Professor, Ruminant Nutrition, *Texas A&M AgriLife Research*

Alternative Feeds: For a Temporary Crisis or Permanent Problem

Dr. Whitney is an Associate Professor, Ruminant Nutritionist, and project leader for the Texas A&M AgriLife Research Nutrition Program, San Angelo. He is also a faculty member in the Department of Animal Science (College Station). He received a B.S. in Animal Science from Southwest Texas State Univ., a master's in Agricultural Education from Texas A&M Univ., and a doctorate in Animal Science/Ruminant Nutrition from the Univ. of Arizona. After receiving his Master's degree, he taught Animal Science classes at Palo Alto College (San Antonio) and did postdoctoral research at Montana State Univ. prior to his appointment in San Angelo. His interdisciplinary research program is directed toward helping producers (especially in the Edward's Plateau Region of Texas) make informed management decisions related to feeding livestock. His primary objective is to reduce feed costs by (1) increasing livestock production efficiency; (2) increasing the value of underutilized feed sources such as dried distillers grains and ground juniper and mesquite trees; (3) using plant secondary compounds to enhance ruminal function, bypass protein, and animal health, and reduce internal parasite viability. Dr. Whitney is currently Chairman of the American Society of Animal Science Western Section committee and a member of the following: American Society of Animal Science, Society of Range Management, American Registry of Professional Animal Scientists, Texas Sheep and Goat Raisers, TX A&M Agriculture Animal Care and Use Committee, TX A&M Council of Principal Investigators, WERA039 research group, and Texas Forage and Beef Workers group.

Rachel Frost

Research Scientist, Range Science, *Montana State University*

Alternative Grazing Strategies for Industry Diversification and Rangeland Improvement

Rachel Frost was raised on a sheep and cattle operation in central Texas and obtained a B.S and M.S in Animal Science from Angelo State University. Her research focused on the influence of genetics and experiences early in life on consumption of bitterweed by sheep. She received her Ph.D. at the University of Idaho after exploring the effects of age and body condition on the consumption of toxic compounds by goats. Her current research focuses on identifying strategies for improving rangeland through targeted livestock grazing and integration of targeted grazing with other vegetation management tools.

Keith Inskeep

Professor, Animal Reproduction and Management, *West Virginia University*

Factors Related to the Ewe That Affect Prolificacy in Sheep

Keith Inskeep grew up on a diversified livestock, dairy and poultry farm near Medley, West Virginia. He completed a B.S. in dairy science (1959) at West Virginia University, an M.S. in genetics (1960) and a Ph.D. in endocrinology (1964) at the University of Wisconsin, under the guidance of a famous reproductive physiologist, Dr. L. E. Casida. He became an Assistant Professor at West Virginia University in 1964 and has spent his entire career there, becoming Professor in 1974. Keith's research has centered upon understanding the mechanisms that regulate reproductive cycles in ruminant females, and applying that knowledge to management of reproduction in sheep, beef cattle and dairy cattle. Currently, he studies factors affecting late embryonic and early fetal losses of potential offspring in sheep and dairy cows. Keith was one of the initiators of the Allegheny Highlands Project, transferring technology to 85 beef and sheep producers in the 1970's and co-authored the proposals for funding of the WV Small Ruminant Management Project, now in its 15th year.

Montana Wool Growers Association

Panel Review and Discussion

Brent Roeder (executive secretary MWGA) and other members of the Montana Wool Growers Association will lead a panel discussion on research accomplishments and what research is needed by the sheep industry to sustain a viable future.

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The Sheep Symposium is sponsored by the following:

*WERA-39 Western Extension, research, and academic coordinating committee, sheep

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*Montana State University Department of Animal and Range Sciences

*Montana Woolgrowers Association

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2013 Sheep Symposium[1]
KEYNOTE ADDRESS: IMPACT OF RESEARCH ON THE SHEEP INDUSTRY

R. W. Kott[2]
Department of Animal Sciences, Montana State University, Bozeman

ABSTRACT: Research, teaching, and extension have had a critical role in the sustainability of the U.S. sheep industry. The purpose of this presentation is to demonstrate how diligent research and education efforts have improved the productivity and quality of the U.S. sheep industry. Specifically addressed will be the development and implementation of 3 successful Montana programs (core testing, winter nutrition and sheep selection), which have had and continue to have dramatic effects on the Montana and U.S. sheep industries. These programs are examples of sound, university-based research and education efforts. Each university represented at this conference could probably identify similar efforts in their own programs. As we review the evolution and implementation of these programs, several key consistent trends seem to emerge. Specifically, the programs: (1) are long-term (8 to 10 plus yr) projects that involve cooperation of numerous researchers and personnel from many institutions; (2) specifically address high-priority issues of the industry and readily involve industry at all phases; (3) have an effective, continuing educational component.
Keywords: education, extension, research, sheep industry, wool

Introduction

Research, teaching, and extension have had an important role in the advances made by the sheep industry and are critical to land grant university mission. The sheep industry has historically had a very close and beneficial relationship between the production industry and USDA and state university research and extension. Early accounts of many producer meetings elaborate the sheep producers' concern for the control and treatment of diseases, wool preparation and marketing, as well as breed improvement and nutrition. This engagement of the industry and federal and state research and extension is evident today and can probably be credited in part to those researchers and educators of the past (e.g., J. L. Van Horn and James Drummond in Montana, P. E Neale in New Mexico, Bob Burns and Alex Johnson at the Wyoming Wool Laboratory, Clair Terrill and Elroy Pohle at USDA and M. M Shelton in Texas, just to name a few). For this paper, I have focused on 3 extremely successful programs (core testing, sheep selection and winter nutrition) that have had dramatic effects on the sheep industry. As we outline the development and implementation of these programs, I think you will see several themes evolve. Each program has had strong formal and informal producer involvement from the beginning, and all had a significant producer education component. In addition, these efforts were long term in nature, with additional research and educational efforts designed to address emerging issues, thus facilitating adoption. There was no ownership of these projects, as each participating group (researcher, extension specialists, and producers) shared the success of the program.

Wool Core Test

Grease wool contains varying amounts of non-wool substances (grease-lanolin, dried perspiration, dirt and vegetable matter) and these substances contribute very little to a fleece's or a clip's value. The amount or percentage of non-fiber material, called shrinkage, could vary substantially between clips and within the same clip between years. In the 1930's and 1940's, it became a standard figure that shrinkage of Montana wools was 65%. A good illustration of the importance of shrinkage (or yield as described over the last 20 or 30 yr) is that for every 1% change in shrinkage/yield there is about a $0.005 to 0.01 difference in price per pound of grease wool.

Up until the last 20 to 30 yr, it has been customary for buyers to estimate the probable shrinkage/yield subjectively. Growers historically were forced to accept this traditional practice in belief that experienced wool buyers could accurately estimate shrinkage/yield. In all actuality, these estimates were quite accurate on average over a large number of grower clips, but were frequently considerably over or under estimated on individual clips. The need for an objective method of evaluating wool clips for shrinkage/yield became evident.

In the late 1930's and early 1940's, the USDA in cooperation with state colleges and laboratories, began a multi-year project to develop a testing method for objectively estimating yield or shrinkage. The early research on shrinkage determination was based on hand sampling and proved to be inaccurate. Subsequent research was directed toward developing a mechanical sampling device which could be used to obtain a representative sample from loosely packaged domestic wool bags (Hughes, 1983). A steel tube, about 18 inches long, driven

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by an electric motor, and equipped with removable cutting blades, seemed to be the most promising, but the USDA had no volume to follow through in a commercial scouring plant to compare the sample with the whole clip. In May of 1939, under the direction of G. Curtis Hughes, the decision was made to scour the entire Montana Experiment Station flock along with bags from 20 producers (a carload of wool in each of 3 yr – 1939, 1940 and 1941) as part of this project. The shrinkage results were very revealing for Montana growers. One of the clips had shrinkage over 65%. The range was from 48% to 67%, or clean fiber yield of 52% to 33%.

The 29th Montana legislative assembly in 1945 passed legislation establishing the Montana Wool Laboratory at Montana State College (currently Montana State University). It was established by the sheep producers of Montana to serve the industry. G. Curtis Hughes was the first supervisor of the laboratory. Initially, the primary purpose of the Montana Wool Laboratory was to develop a test procedure for supplying basic information for selling a clip on its merits. That program involved collaboration with USDA and state university laboratories in Wyoming, Colorado, Idaho, New Mexico, Utah and California.

The first core samples were scoured in the newly constructed laboratory in 1947. Mr. Hughes resigned (to return to the family ranch) and was replaced by James Drummond. James Bassett (who later became the head of wool research at Texas A & M University); Kenneth Colman (who later headed the work at the Montana Wool Laboratory) and Robert Stobart (who later became head of wool research in Wyoming) joined the staff. Core testing research and extension efforts continued through the 1950's and 1960's. It was reported that in 1 yr in the mid 50's the laboratory core tested 1.4 million pounds of Montana wools. Now used overseas by major wool producing countries, core testing of shorn wool is common practice throughout the world.

In recent years, the Montana Wool Laboratory has focused research efforts towards developing strategies for objectively evaluating wool characteristics on individual sheep for clip preparation and selection purposes. The Montana purebred industries have benefitted by raising rams within a breed for more uniformity of wool grade, longer staple length and more desirable color. That has spread throughout the commercial industry. Today, Montana enjoys the reputation of producing some of the finest quality wool in the U.S. The activities of the Montana Wool Laboratory certainly played an important role in the progression of the wool industry in Montana.

Winter Nutrition Programs

In the 1950's, extensive work was done on defining appropriate winter supplementation programs for ewes grazing on native ranges in the Intermountain West. P. Orcutt (1956) summarized the Montana work in a number of extension sheep presentations and field day reports. He generalized that in 1 out of 4 yr, ewes receiving no supplement performed as well as supplemented ewes; 2 out of 4 yr, energy supplemented ewes performed better than non-supplemented ewes, but similar to protein supplemented ewes; and in 1 out of 4 yr, protein supplemented ewes performed best. Over the 4-yr period, the energy-supplemented ewes produced 9.4 more pounds of lamb per year than did non-supplemented ewes. In addition, it was documented that there was no advantage to feeding over 1/3 pound per day of supplement. D.C. Clanton (Ph.D. dissertation) in Utah indicated that a protein pellet improved lamb production over no supplement or an energy pellet. Thomas (from 1984 to 1993) reported that ewes consumed (selected or grazed) diets containing approximately 7.0% CP in 3 out of 4 yr. However, in 1 of the 4 yr, the protein content decreased 4.5% and probably explained much of the variation in results between an energy and protein supplement. The energy supplement was probably adequate in those years when CP in the diet was around 7%.

The early research conducted in the 1950's by J. L. Van Horn, E. P. Orcott, D. C. Clanton and later by V. Thomas and coworkers has provided the basis for winter supplementation projects (summarized by Thomas and Kott, 1995). General conclusions are:

1. Supplementation is cost effective most winters in Montana.
2. Supplements fed at 0.25 to 0.33 pounds per head per day, or 0.2 to 0.3% of ewe body weight, did not reduce forage consumption, and environmental factors influenced forage intake more than supplement type.
3. Body condition entering the winter influences productivity and the response to supplementation. Ewes can lose some body condition (0.3 to 0.4 units of a body condition score) during the winter if they enter the winter in good body condition.
4. Ewes can be supplemented on alternate days while grazing winter range.
5. Energy supplements are cost effective when ewes can afford to lose some body weight, range forage is available and winter weather is mild.
6. Protein supplements are cost effective when ewes cannot afford to lose body weight and need to gain weight or when winter weather reduces forage intake. Protein supplements should provide a minimum of 0.07 pounds of CP per day.

Sheep Selection Programs

Much of the basis for our current sheep selection programs for range sheep come from work conducted at the Western Sheep Breeding Laboratory (USDA, ARS, U.S. Sheep Experiment Station at Dubois, ID; Nordby, 1939) and collaborators in Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Texas, Utah, Washington and Wyoming. The U.S. Sheep Experiment Station was established in 1916 and the activities of the Western Sheep Breeding Laboratory were most active during the 1940's. Research included the genetic relationships of weaning traits, wool traits, color on legs, face covering and neck folds. In addition, they evaluated the heritability of body condition and type, jaw deformity (over and under shot jaws) and the relationship between weaning and yearling weights. In later years, they

highlighted the importance of reproductive performance and crossbreeding in range ewes. Much of their work culminated in the development of the Columbia (1941) and Targhee (1951) breeds of sheep (Drummond, 1983). Today, these two breeds play an important role in range sheep production in the West. In 1958, P.E. Neale (New Mexico) developed a selection scheme dividing the herd into sub-herds of different productive levels (Stauder and Neale, 1958). The top or super sub-group comprised the top 10% of the flock. This sub-group can be used to raise replacement sires, allowing the use of highly-productive sheep to the greatest advantage. The next sub-group referred to as the A sub-herd was comprised of 40% of the ewe flock. Most of the ewe lamb replacements would come from the super and the A sub-herds. The B sub-herd comprised 30%. The C sub-herd was comprised of the bottom 20% of the ewe flock and would produce no replacements. Later the Texas program slightly modified this breeding scheme dividing the flock into thirds with all replacements coming from the A & B sub-herd and the C sub-herd being bred to terminal sires.

Central ram tests have been utilized to identify genetically superior sires for outstanding growth and wool characteristics. Texas A &M University, University of Wyoming and North Dakota State University conduct central tests in cooperation with the American Rambouillet Association to identify top performing rams within the breed. It is impossible to quantify the impact these tests have had on the industry, but it's safe to say that it's been substantial. Montana State University began a central ram test in 1981, but participants in that test have declined since the adaption of the National Sheep Improvement Program (NSIP). New Mexico State University conducted a range performance test of rams where measurements were taken over a one-year period but rams were maintained under range conditions. North Dakota State has conducted a terminal sire test in previous years. These tests have benefitted the Industry in two ways. First, they have assisted the Industry in identifying genetically superior sires that have gone on to have significant genetic impacts on their breeds. Secondly, these tests have played an important role in promoting the awareness among producers on the economic importance of objective measuring of traits within our selection program.

In 1986, the NSIP was developed to provide genetic records to the sheep industry. Recently, that program joined Lamb Plan from Australia. Montana purebred producers have committed to this program. NSIP provided sheep producers a tool that allows them to readily select for reproductive performance. The relative importance of reproduction performance became clearly evident to producers through the development of the Western Range Profitability Index in 2007 (Borg et.al., 2007). Major strides in reproductive performance have occurred since its implementation. In 2009, the development and implementation of ribeye measurements was added. Genetic records are now being incorporated into the sale catalog of the Montana Ram Sale, along with an educational program. MSU is also a consigner to the sale (it has been a ram sale consignor for 63 years) which, has a major affect on the creditability of MSU's research and educational program. Montana's genetic record program is based on the use of the US Range Index which was directly developed by and based on research conducted at MSU. The availability of genetic records has drawn interest from surrounding states. In fact, 36% of the rams went to out of state buyers with availability of extensive records being a major factor stimulating out-of-state interest. The average of this year's sale was $1,100 which was substantially higher than ram sales in surrounding states (sales averaged around $500).

Conclusions

Through the years, the sheep industry has benefitted substantially from land-grand university research, teaching and extension programs, and the USDA. The survival of the industry will be dependent on how the industry adapts to the challenges/opportunities of the future; research and extension will play a vital role in that process. It is critical that industry and academic personnel remain engaged with each other to assure that research and extension efforts are relevant to the industry and there is a path to facilitate adoption and implementation.

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2013 Sheep Symposium[1]
APPLYING GENOMIC SELECTION TECHNOLOGY TO THE SHEEP INDUSTRY[2]

R. R. Cockrum[3,†], K. J. Austin[†], N. K. Pickering[‡], R. M. Anderson[‡], D. L. Hyndman[‡], M. J. Bixley[‡], K. G. Dodds[‡], S. L. Lake[†], R. H. Stobart[†], J. C. McEwan[‡], and K. M. Cammack[†]

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ABSTRACT: Crossbreeding schemes that balance breed complementarity and hybrid vigor promote a more sustainable and consistent product. Incorporating multi-trait selection indices and genotypic analyses within crossbreeding schemes will further improve selection accuracy. Genotypes for both simple and complex traits can be determined using next-generation technology, such as SNP chips. Genotype tests can easily identify deleterious alleles for simple traits, such as scrapie susceptibility and spider lamb syndrome. However, complex traits such as feed efficiency, longevity, and fertility are controlled by complicated gene networks. Unfortunately, these traits are often too difficult or expensive to quantify and(or) cannot be measured until later in life. Because the majority of these traits demonstrate genetic variability, they serve as ideal traits for genomic selection. High-density SNP chips can be used to identify gene regulatory networks and genotypes associated with complex traits. Identified genotypes can be used to construct marker panels to select for economically relevant traits. The same technology can be used to develop molecular breeding values to increase the accuracy of prediction. Pedigrees used to generate existing estimated breeding values (EBV) are often inaccurate and do not account for the variability of genes inherited during fertilization. Therefore, kinships constructed from genotypic relationships provide a more reliable estimate for molecular breeding values. Through improving current practices and incorporating new technologies, sheep producers will be able to improve genetic trends of economically important traits and provide a better product.
Key Words: genetics, estimated breeding values, marker-assisted selection, sheep, technology

Introduction

Agriculture production must increase an estimated 70% to meet the nutritional demands of a 30% growth in world population by 2050. Unfortunately, resources (e.g., land, labor, feeds) that are necessary for agricultural growth are being shifted away from agriculture and towards human use. One solution for the sheep industry to address world food demands is to improve production efficiency, yield, and quality by combining classic breeding selection techniques with emerging genomic selection technologies.

The majority of the sheep industry incorporates crossbreeding to some extent. However, the crossbreeding systems that are typically implemented arbitrarily select animals for mating that result in a poorly structured system and, ultimately, an inconsistent product. Characteristics of an ideal crossbreeding system should include: easy maintenance, breed complementarity, optimized hybrid vigor, and uniform products (Thomas, 2006). Incorporating crossbreeding systems, selection indices, and genotyping for economically relevant traits (ERT) can be used to increase trait selection accuracy.

Multiple-trait selection is best implemented through the use of a selection index (Hazel, 1943), which incorporate pedigree relationships to predict progeny performance for multiple traits. Selection techniques based on observable phenotypes are not as effective in propelling production forward; therefore, selection progress must be measured and quantified using selection indices or breeding values that account for genetic interactions (Figure 1; Dekkers and Hospital, 2002). With the mapping of the ovine genome, technology is now available to begin selecting for complex ERTs. Traits that are difficult or expensive to measure, complex, heritable, and(or) take place later in life are ideal for marker-assisted selection. Traits such as feed efficiency, fertility, longevity, and disease susceptibility are considered complex ERT. As technology continues to develop, it will become more affordable and more accessible for producers.

Crossbreeding

The mating of unrelated individuals that takes advantage of hybrid vigor characterizes crossbreeding systems. A good crossbreeding system balances 4 features: easy to implement and maintain, utilizes breed complementarity, optimizes hybrid vigor, and produces a uniform product. Structured crossbreeding systems are important to take advantage of breed characteristics that complement environmental conditions. No individual breed excels for all desired traits, and specific performance traits

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(i.e. maternal, carcass, or growth) are emphasized in certain breeds; therefore, crossbreeding systems are necessary to maximize production objectives (Leymaster, 2002). Many crossbreeding systems for sheep are currently in place that maximize both heterosis and breed complementarity, including terminal crossing, rotational crossing, and composite breeds. For example, in the Western U.S., a 3-breed rotational system using Rambouillet, Columbia, Targhee breeds would emphasize environment adaptability, longevity, and fleece characteristics, but may be deficient in growth and prolificacy (Thomas, 2006). Though incorporating mating systems into current management practices will take advantage of hybrid vigor and reduce the prevalence of deleterious alleles, it is also important to make mating decisions based on quantitative information that can be traced to improve flock genetic progress.

Index Selection

A selection index is used for genetic prediction to combine traits to select animals in an optimal way. Through combining all of the available information on an individual and its relatives, a breeding value can be estimated as:

$$I = b_1x_1 + b_2x_2 + \cdots + b_nx_n$$

where I is the genetic prediction, b_i is a weighting factor, x_i is the performance record, and n is the total number of observations for that record (Mrode, 2000). Selection indices can be constructed for a variety of purposes. The most basic index is an estimate of breeding value calculated for a single trait using a single piece of information on an individual animal. Alternatively, using the same methodology, index-breeding values can be calculated for multiple traits using records of an individual and its relatives (Henderson, 1975). Selection for reproductive rate using breeding values in Rambouillet sheep was estimated to increase lambing rate by 16% over a 20-yr period (Burfening et al., 1993). Variables such as genetic variation, accuracy of selection, selection intensity, and generation interval can all impact genetic change.

Next-generation Sequencing

Marker panels are now available for sheep (i.e., Ovine SNP50 BeadChip) that use thousands of SNP (single nucleotide polymorphisms) or genotypes to select for health, performance, wool, and carcass traits. The Illumina Ovine SNP50 BeadChip was developed as a concerted effort by researchers at AgResearch (New Zealand), Baylor UCSC, CSIRO (Australia), Illumina, and the USDA. The International Sheep Genomic Consortium (ISGC) is currently developing a SNP chip capable of genotyping up to 700,000 loci, which is expected to be commercially available late 2013. The Illumina Ovine SNP50 BeadChip was constructed from sequencing Romney, Texel, Scottish Blackface, Merino, Poll Dorset, and Awassi ewes. The development of genomic technologies are being used to investigate disease and toxin resistance, parasite resistance, reproductive efficiency, longevity, milk traits, and feed efficiency in livestock. Genomic relationships between markers and ERTs can be used to develop marker panels for marker-assisted selection and molecular breeding values.

Applications

Multiple genotype tests are commercially available in sheep to determine disease susceptibility to disorders such as scrapie and spider lamb syndrome. Scrapie is a prion disease in sheep that depends on three codons (171, 154, and 136), with codon 171 the major determinant of disease susceptibility. Sheep with glutamine (QQ) at the 171 position are considered susceptible to developing scrapie and can pass down the susceptibility to the disease to their progeny (Clouscard et al., 1995). Alternatively, spider lamb syndrome is a semi-lethal homozygous recessive disorder that results in skeletal deformities. Lambs with 2 copies of the mutation within fibroblast growth factor receptor 3 (FGFR3) will result in gene inactivation, which impairs cartilage development into bone (Cockett et al., 1999). Both scrapie and spider lamb syndrome are considered simple traits that can easily be selected against using genotype technology.

Intricate gene networks regulate complex traits such as feed efficiency, fertility, and longevity making them more difficult to observe improvement using traditional selection techniques. As genome-wide association technology, genetic information that spans the whole genome, continues to develop so does the ability to identify gene networks involved in complex traits. For example, researchers at AgResearch in New Zealand and the University of Wyoming recently collaborated to identify genotypes associated with residual feed intake (RFI), or feed efficiency in sheep using the Illumina Ovine SNP50 BeadChip (Cockrum et al., 2012). Residual feed intake is an ideal trait for marker-assisted selection because it is moderately heritable (0.16 to 0.43), labor intensive, and expensive to measure. Individual feed intake measurements were collected from five performance tests for dual-purpose (n = 183) and meat breed (n = 147) rams at the University of Wyoming using the GrowSafe System. Residual feed intake, the difference between actual feed intake and predicted feed intake, was estimated within each test for each ram. Genotypes were determined using DNA collected from both the dual-purpose and meat breed rams using the Illumina Ovine SNP50 BeadChip. A simple fixed association test was performed with each SNP included as an additive effect to the residuals from a polygenic model. Fixed effects included birth type (single, twin, triplet), birth year, flock, and contemporary group. Kinship relationships using genomic relationships indicated that rams had an average inbreeding coefficient of 9.6%. Based on genotypes, RFI was 14% heritable, and 24 of the 50,896 SNP reached nominal threshold levels of $P < 1.00^{-03}$. Chromosomes 3, 9, and X contained the majority of associations with ≥ 3 SNPs that reached threshold. Genotypes were mapped against the ISGC Ovis_aries_1.0/oviAri1 (i.e. ovine genome annotation version 1.0) assembly to identify genes associated with RFI. Ten genes close to the identified associated SNPs, suggested a relationship with the genes and residual feed intake. Functional categories associated with the 10 genes

included regulation of embryonic development, neurogenerative processes, and protein coding. Though research is still being conducted on genomic relationships with feed efficiency in sheep and needs several thousand animals to provide reasonable molecular breeding value accuracy, similar research has been conducted for several other traits in sheep.

Currently, Pfizer Animal Health (Zoetis) has a marker panel, Sheep50K, available in New Zealand with genomic breeding values available for 22 traits (Auvray et al., 2011). The marker panel is applicable for Romney, Coopworth, and Perendale breeds > 30% composition, and is derived from the Illumina Ovine SNP50 BeadChip. Through incorporating traditional and contemporary mating and selection decisions, genetic progress for ERTs can be more accurately measured subsequently increasing site-specific production and/or profitability.

Commercial and Economic Information

The Sheep50K is available commercially through Pfizer Animal Health (Zoetis) in New Zealand (http://www.pfizeranimalgenetics.co.nz/sites/PAG/nz/Pages/Testing_and_Results.aspx). Currently, the Sheep50K test costs approximately $17.00 (USD) for DNA extraction and $350.00 (USD) for genotyping per sample. Blood cards, semen, tissue, whole blood, or hair follicle samples can be used as DNA sources. Traits predicted on the Sheep50K include production, wool, meat yield, and health (i.e. carcass weight, lamb weaning weight, loin eye area, live lambs at birth, 12-mo greasy fleece weight, greasy lamb fleece weight, fat yield, lean yield, fecal egg count, facial eczema, etc.). GeneSeek (Neogen Corporation, Lincoln, NE) offers DNA extraction for approximately $3.00 USD per sample and whole-genome analysis on the Ovine SNP50 BeadChip for < $0.001 (USD) per data point [$60.00 (USD) per sample]. However, individuals are responsible for the computationally intensive data analyses. Most land grant universities have access to large servers and employ biostatisticians that can assist in the analysis of large datasets. The GeneSeek website, http://www.neogen.com/GeneSeek/SNP_Illumina.html, provides additional information on the Illumina Ovine SNP50 BeadChip.

Conclusion

Greater production that is needed to feed a growing human population requires producers to improve current mating selection strategies and incorporate technologies to target more complex traits such as feed efficiency. As whole-genome sequencing techniques improve, generating molecular breeding values for ERTs will become more affordable for the sheep industry. Currently, costs associated with whole-genome sequencing in the U.S. are too great to warrant the expense by individual producers, but may be useful for breed associations and researchers. Developing marker panels for disease resistance, feed efficiency, wool, and carcass traits through whole-genome sequencing will provide the sheep industry a useful, inexpensive genomic selection tool. However, researchers and producers must collaborate to have enough animals (n > 2,000) to develop these tools. Furthermore, researchers must implement translational research to bridge the gap between science and application.

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2013 Sheep Symposium[1]
SHEEP PARASITES: PROBLEMS, RESISTANCE, NEW PRODUCTS AND PRACTICES FOR PARASITE CONTROL

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ABSTRACT: Parasitic disease in sheep can be a major or a nonexistent problem in a herd depending on where the sheep are raised and how they are managed. Because of this variability there is no one approach which will aid all producers in preventing disease or in limiting its effects if disease occurs. However there are considerations that may aid in developing an approach to controlling disease. Parasitic infection does not equal disease as sheep are able to tolerate some level of infection without any adverse effects. In fact, exposure to a limited number of parasites may stimulate a protective immune response. The ability to develop protective immunity depends on age, breed, reproductive and nutritional status and is variable among members of the flock. The level of exposure and the level of acquired immunity vary with the time of year, how long the pasture was rested and who else shared what other species of grazing animals share the pasture rendering the forage either relatively safe or extremely dangerous to animals entering the pasture. The idea that the entire flock requires deworming at regular intervals is not supported by research. Instead selective use of anthelmintics (dewormers) and management of the flock by understanding how parasites make a living may help control parasitic disease.
Keywords: anthelmintic, management, parasite, resistance, sheep

Introduction

The approach to controlling parasites depends largely on where you live, what classes of livestock are raised, how they are managed and perhaps, most importantly, where the parasites came from. Did you buy sheep with resistant worms in them, especially species of parasites that survive in your environment? Because of these differences there is no answer that fits all situations and these variations must be considered when developing your strategy. Although important parasitic protozoan agents and arthropods infect or infest sheep, only disease caused by nematode helminths (worms) will be discussed.

The life cycle of the economically important nematodes is similar; the adult worms live in the digestive tract with each species occupying a specific portion. These adult worms mate and produce eggs that, for the most part, look just the same if they came from worms in the abomasum, small or large intestine. The eggs are passed in the feces then larvate and hatch in a few days to weeks, depending on temperature (the warmer the temperature the faster the development with no development at or below freezing). The larva hatches from the egg then feeds on fecal bacteria and eventually molts to the infective stage, which is encased in a sheath that protects it from drying out. The infective larva exits the fecal pellet and ascends on vegetation in a film of water (dew or rain) where it is grazed by a sheep. Development to the infective stage takes a week to a month, again depending on temperature. The feeding larvae are easily killed by drying out but the infective larvae survive in the pasture for a month to a year, again depending on temperature. Elevated temperatures speed up all stages in the environment and low temperatures prolong development and survival.

When the larva is ingested by the sheep it enters the rumen where it loses the sheath then goes to the organ of predilection. There the larva enters the lining of the organ such as a gastric or intestinal gland where it rapidly molts to the next larval stage. This forth stage larva begins to feed and develop, eventually to adults, or undergoes arrested development in the early fourth stage. One genus *Oesophagostomum* migrates deeply into the intestinal wall and migrates for a considerable time. Most species emerge from the lining of the organ and become adults who mate and begin producing eggs three to four weeks after entering the sheep. The adult worms may live for months with each female worm producing 25 to 5,000 eggs / d depending on worm species.

When you think about where and how sheep are raised there are many factors involved. Are you in a locality usually receiving 6 or 60 inches of annual rainfall? Do you irrigate or rely on natural precipitation? Is the growing season 7 or 12 mo long? Where do the sheep feed? Are the sheep fed or grazed in a pasture, is the feed in a feed bunk or on the ground, or in different places at different times depending on weather/climate conditions? Is the stocking rate 60 ewes/section, or 6 ewes/acre? Are pastures rotated and the sheep grazed for intervals of 5 d or 5 wk? Are the sheep overwintered in a dry lot or barn, on pasture or on a dormant hay field? When is the lambing season? When are the lambs weaned? Are the pastures shared or rotated with cattle, goats, horses or others such as white-

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tailed deer or wapiti? Is there a history of liver flukes on the property? The answers to these questions will begin to give you an idea if there may be a parasite problem in your flock.

Parasitic Disease vs. Infection

An important concept to consider is the difference between infection by parasites and disease caused by them. Worms may infect sheep with little or no signs of disease, but when present in large numbers cause severe disease or death. Therefore, controlling parasite numbers is important because a few worms may stimulate a protective immune response but a high population can cause disease. The exception to this is a single deer fluke (*Fascioloides magna*) or only a few meningeal worms (*Parelaphostrongylus tenuis*) may kill a sheep. With other worm species it may require thousands to cause diarrhea, or a thousand adult *Haemonchus* to exsanguinate a sheep. Still others such as tapeworms, which are large and disgusting, are not likely to ever cause disease without help from other agents.

Origination and Adaptation

Most of the parasites that sheep acquire in North America came here with imported sheep or other ruminants. A few parasites were already here in hosts such as white-tailed deer. Over time the parasites spread to new areas with the hosts (i.e. sheep and deer). Some of the imported worms were brought into geographic areas similar to that from whence they originated and those parasites found a very hospitable environment and have thrived. Worms that originated from areas where conditions were hospitable but were then imported to one where it was too cold, too hot or too dry for prolonged periods, either adapted to the local conditions or never established. An example of adaptation is the tropical parasite, *Haemonchus contortus* which is able to survive in cold climates because the majority of worms acquired from the pasture during the autumn, undergo arrested development. That is, the worms do not develop into mature breeding adults. Instead, the larval worms burrow into the lining of the abomasum and cease development for a time, not feeding or reproducing. Because they are metabolically inactive, they are not detected by the immune system. They later emerge and mature just before the green up of pastures or at the time of lambing because their offspring would not survive winter in a pasture or barn. How do these worms know it is spring? They are receiving signals via the sheep's pineal gland that indicates the days are getting longer and/or the hormones of the ewe's lactation kick in and the worms know it's time to reproduce.

Generally speaking, the gastrointestinal nematode parasites of sheep can be divided into two groups: warm season parasites, which can be transmitted year around in the deep south but only during the summer in the north, and cool season parasites, which can be transmitted during the winter in the south and elsewhere when the temperature is above 50º F. Cool season parasite larvae on pasture do not survive a long hot summer, so may go into summer arrest inside the sheep and emerge in the autumn. One genus, *Nematodirus,* is adapted for harsh climates. Larval development occurs within the egg shell, which protects the larva from cold or desiccation. Knowing which parasites are likely to cause problems in your sheep is essential for long term control programs on your property.

Immunity to Parasites

There are a few practices that can be used in many flocks to help control internal parasites. One thing to keep in mind is that different parasites, and different breeds of sheep, originated in different geographic regions. Those breeds of sheep that survived for generations in a defined geographic region are likely to have evolved a level of resistance or tolerance to the worm species originating in that area. For instance, *Haemonchus contortus* is a tropical worm and breeds of sheep developed in the humid tropics are much less likely to suffer from disease caused by this parasite (Amarante et al. 1999). By the same token, tropical sheep may be more susceptible to worms imported from Northern Europe.

Lambs, even in resistant breeds, exposed to parasites are usually unable to manifest resistance to worms until they are 4 to 6 months of age. This period allows the immune system time to mature and become resistant to worms. Older sheep with a functional immune system that have been exposed to worms still may not be able to express their resistance if they are stressed, on a low protein diet, or during early lactation. Within a flock, the numbers of worms in individuals varies considerably, even among ewes consuming the same diet. We find that 20% of the flock have 80% of the worms and of course are producing the greatest numbers of worm eggs. When individual sheep are identified that constantly have a heavy worm burden they should be removed before they die of disease or pass on more worms to the flock (Kelly et al., 2013).

During lambing ewes have a rise in fecal worm egg count because the hormones associated with lactation suppress the immune response against worms. This is called the periparturient rise (Dunn, 1978). The periparturient rise is more pronounced in younger ewes that are still growing and feeding their young and in older ewes with twins or triplets who cannot keep up with the demands of their lambs and sustain their own needs. The effect of the periparturient rise is usually insufficient to cause disease in ewes but ensures that the eggs that are produced hatch and the larvae develop to the infective stage when the lambs begin to nibble forage. In addition to the parturient lack of resistance, arrested larvae that were discussed earlier, acquired from pasture during the autumn begin to develop to the adult stage with the advent of spring and the ewe is unable to rid herself of these worms during early lactation.

Management to Lesson Exposure to Helminths

Limiting the numbers of worms to which a sheep is exposed can be accomplished either by removing the adult worms from sheep, by use of an anthelmintic or by managed grazing. When parasite eggs are deposited in pasture it takes at least a week with plentiful rainfall in the

middle of the summer to develop to an infective larva, therefore rotation of pastures may avoid larval contamination. However once an infective larva is on the pasture, if the conditions are not suitable for transmission, it may survive for months in the soil and may overwinter. Therefore, a pasture must be rested at least a month in the humid tropics, but it takes 3 mo or longer during other seasons to lessen the numbers of pasture larvae (Colvin et al., 2012; Craig and Miller, 1990; Craig, 2006; Dobson et al., 2011; Dunn, 1978; Eysker et al., 2005). The length of time larvae survive on pasture is influenced by the moisture required for the worms to escape the fecal pellet and ascend vegetation (O'Connor et al., 2008). Sheep grazing mountain meadows where they are moved frequently ensures that, even if worm eggs are deposited on the pastures, the sheep avoid meaningful numbers of parasites unless they are bedded in the same area for a long time. Another approach that may aid in lessening the numbers of parasite larvae in pastures is by using the pasture for grazing of animals that are resistant to worms such as older non-lactating sheep, adult cattle (Bailey et al., 2009), and horses or after harvesting hay so that at-risk animals can safely graze.

During periods of drought, infective parasite larvae remain in the fecal pellet or move underground, and are not picked up by grazing. When the rains begin these larvae rapidly move out of the feces or soil onto vegetation. The vegetation near where the feces were deposited and the rapid growing green grass has many larvae which are acquired by the grazing animal. Treating the recently exposed animals 2 to 3 wk after the onset of the rains may remove the newly acquired worms before they have an opportunity to reproduce. This tactical approach to deworming may prevent problems a month or two in the future but may be a selection mechanism for anthelmintic resistant worms.

Treating ewes at or near the time of parturition with an effective anthelmintic lowers the numbers of larvae on the pasture and in some geographic areas may be the only deworming required for the entire year. However, in high rainfall intensive grazing situations it is a selection mechanism for resistant worms (Leathwick et al., 2006). Another approach is increasing the protein content of the diet at lambing, which aids in lessening the success of the worms within the ewe, and therefore, they do not contaminate the pastures with as many eggs. However, the use of an anthelmintic before turning animals onto a parasite free pasture ensures that only worms the flock is exposed to a month later are the offspring of the survivors. So, one approach should be to expose at risk animals to pastures with low numbers of parasite larvae so the immune response is stimulated but no disease seen, or leave some of the adult sheep untreated (Dobson et al., 2011). The worms in the untreated sheep reproduce and their offspring mate with the offspring from the treated sheep diluting the genes for resistance (the refugia) in the next generation of parasites.

In addition to management several approaches other than anthelmintics may prevent disease caused by gastrointestinal worms. Copper oxide wires may be an effective way of protecting adult sheep from *Haemonchus* but can also cause chronic copper poisoning if not carefully managed. In some situations the use of fungi that kill newly hatched nematode larvae can lower the pasture contamination. A fungus, *Duddingtonia flagrans*, traps nematode larvae with its mycelia then enters the larval worms and devours them. Its spores pass through the sheep digestive tract and are used to control parasites in some areas in Europe and have been evaluated in North America for control of larval nematodes in several species of grazing animals. However, these fungi do not kill worms in the digestive tract of the sheep so must be constantly fed or incorporated in a slow release ruminal bolus to make any long term impact (Fleming et al., 2006).

Plants containing high levels of tannins, such as serecia lespedeza or juniper, may be grazed or fed to affect the newly acquired larval worms before they can establish in the gastrointestinal tract. These plants can also provide high quality protein. In areas where these plants grow well, they can be a major aid in parasite control (Terrill et al., 2012). Elsewhere they may be offered as hay or pellets but if the pastures are rapidly growing are unlikely to be consumed in high enough quantities to prevent disease.

Resistance to Anthelmintics

Resistance to deworming agents is not a matter of if, but of when. Essentially, there is a direct relationship between the number of times a worm population is exposed to an anthelmintic and the advent of resistance as a clinical problem. When an anthelmintic is used and it does not kill 100% of the targeted worms, the survivors probably are carriers of resistant genes. The survivors have no one else to mate with but another resistant worm.

Under dosing is a powerful selection mechanism for resistance (Smith et al., 1999). If the average weight is used as the dose for the flock, then an under dose is administered to the larger animals. One practice that simulates under dosage is the subcutaneous administration of cattle dewormers in small ruminants. Initially the blood levels are high then drop off. In cattle this long residual effect kills incoming worms from weeks to months following its use, depending on the product and worm species. In sheep, and to a lesser extent in cattle, as the blood levels drop, the drug then removes only the worms without genes for resistance; in effect, acting as a cutting gate turning back the wimps and letting the super worms in (Craig and Miller, 1990). The sheep and goat industry did a wonderful job of selecting for ivermectin resistance by this practice.

The most obvious way of preventing selection for resistance is by switching anthelmintics, i.e. rotating drugs. Unfortunately, the obvious way is wrong and the rotation of drugs within a grazing season selects for resistance to all of the drugs in the rotation. This resistance will occur with different drugs at different time intervals, but ensures that multiple drug resistance will occur faster than if a drug was used until no longer effective before switching to an effective drug. A more effective method to slow anthelmintic resistance is yearly rotation with an anthelmintic from a different family; the worms at the beginning of the year are the survivors of last year's

treatment regimen and may be susceptible to next year's anthelmintic.

Discovering anthelmintic resistance at the farm or ranch is often made only after a drug completely fails and animals sicken or die. There may be a clinical response from an anthelmintic with only 40% to 50% efficacy but this leads to massive pasture buildup of resistant worms, and numbers win. The fecal egg reduction test (FERT) is the most common method of evaluating a herd or flock. Samples are taken from several (6 to 20) individual animals at the time of treatment and again in 10 to 14 d and a comparison of egg counts before and after treatment are made. Unless there is a 95% or greater reduction in egg count you do not have a truly effective drug against the adult nematodes present. But, certainly a 93% or 85% reduction is better than a 20 to 50% reduction, even if it is not ideal. Several laboratory tests for resistance have been developed. Larval development assays in which nematode eggs are placed in solutions containing varying concentrations of anthelmintics are evaluated for the ability of the worms to hatch then develop to the infective stage (Taylor, 1990). Even small populations of resistant worms can be detected by this system. It can be compared to the FERT to determine if insufficient dose was administered in a particular flock. However, the assays are time consuming and expensive to run.

The effectiveness of dewormers may be increased by some of the following strategies. Fasting overnight before administering a benzimidazole (white drench) or by treatment over a period of several days enhances efficacy of this class of drug. Incorporating the drug in palatable feed and making sure that all animals have access to and then consume it may accomplish deworming without hands-on treatment (Craig, 2006). In general, the use of blocks or minerals to administer drugs has been disappointing as consumption is too variable to ensure that all the hosts have adequate intake each day. This hit or miss situation is likely to lead to low dose selection for resistance.

When a premise has been identified where multiple anthelmintic resistances occurs then combinations of anthelmintics from different families may be useful (Bartram et al., 2012; Colvin et al., 2012; Craig and Miller, 1990; Craig, 2006; Dobson et al., 2011; Dunn, 1978; Eysker et al., 2005; Fleming et al., 2006; Kelly et al., 2013; Kenyon et al., 2009; Kenyon and Jackson, 2012; Leathwick et al., 2006; Miller and Craig, 1996). Even though each individual anthelmintic is ineffective, combining drugs at full therapeutic levels may be effective. The combinations must be drugs from different classes with different mechanisms of action. The combination of a benzimidazole or a macrolide and levamisole has been very successful in controlling worms in small ruminants in Australia and in some, but not all, flocks in Texas. Of course this approach is destined to fail in time.

When benzimidazole resistance is diagnosed in a flock, the worms remain resistant over an extended period of time. The benzimidazole resistant worm (*Haemonchus contortus* variety *tejano*) is truly a super worm. It drinks more blood, lays more eggs and appears to survive better in the environment. The prepatent period (from larval entry into the sheep until eggs are found in the feces) is a bit longer than other strains. Sheep with Texas *Haemonchus*, not exposed to benzimidazoles for 15 yr, were treated and there was only a 50% reduction in FERT. However, levamisole resistance is a recessive trait. Therefore, if levamisole resistance is detected, by not using the product from 3 to 6 yr may lead to a worm population again susceptible to the drug. It appears that some ivermectin resistant strains of *Haemonchus* lack cold tolerance during the free-living stages. Where there are prolonged periods of low temperatures the larvae die. This may be one reason that ivermectin resistance is not as common in the northern United States.

Two families of anthelmintics, not yet on the market in the United States, are used in other countries and one is being evaluated for use in sheep in the United States. Because they are chemicals completely different from the available products, they are likely to be effective where others have failed. Hopefully, the prudent producer will not use the new product unless there has been failure with the currently used drugs and then use it to prevent disease not get rid of all parasites.

Targeted or selective deworming, that is treating only the sheep at risk, such as early lactation with high milk producing females, young animals in a herd, or individuals with signs of disease (pale mucous membranes, high egg counts, bottle jaw), lowers the chances of selecting resistant parasite because the worms in the non-treated animals will survive whether possessing resistant genes or not (the refugia) (Kenyon et al., 2009; Kenyon and Jackson, 2012). The refugia is a population of worms that have not been exposed to a specific anthelminthic and their offspring will be in the pastures to be grazed as will the survivor offspring of the resistant worms. The theory, with data to support, is that the resistant and susceptible worms will mate diluting the effect of the resistant genes. This approach is labor intensive and may require frequent observations. When a large number of animals are showing signs of disease or in large flocks where observation of each individual animal is too time consuming leaving 1 to 4% of the animals untreated enables a sufficient number of survivor worms so that selection for resistance is minimal (Dobson et al., 2011).

FAMACHA, an approach to identify sheep for targeted treatment, is where ocular mucous membrane color is evaluated with a chart to determine the degree of anemia. This methodology is effective where *Haemonchus* is the predominant helminth. The idea is to determine the individual animals with the largest numbers of worms and only treat them. This removes most of the source of pasture contamination but puts no selective pressure on the worms in the remainder of the flock, the refugia worms (Miller et al., 2011; Molento et al., 2009). The chart can also be used to evaluate the flock to see how things are going before clinical signs are seen. The greatest problem with the FAMACHA approach is the lack of susceptible worms to any anthelmintic on some ranches.

Resistant worms are present inside a host that has been treated with an anthelmintic that has removed the susceptible worms. Maintaining newly purchased animals in a barn or dry lot until they have been evaluated after treatment with an effective anthelmintic or combination of

anthelmintics is an essential part of a herd health management. Many producers isolate animals that have evidence of some infections so they don't have a chance to infect the remainder of the flock but forget that resistant worms in a healthy animal will infect the flock if not this year then the next.

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2013 Sheep Symposium[1]
FACTORS THAT AFFECT PROLIFICACY IN SHEEP[2]

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ABSTRACT: Prolificacy is the end product of number of ovulations × fertilization rate percentage × embryonic survival percentage × fetal survival percentage × survival of the birth process percentage; it is expressed as lambs born per ewe lambing. In the ewe kept for meat production, it is the single most important trait in determining income from the enterprise. Prolificacy has been considered a lowly heritable trait, but selection can be effective, especially if estimated breeding values based upon performance of the individual and relatives are available. It is influenced by specific gene mutations that involve bone morphogenetic protein-15 and its receptors and growth differentiation factor-9. These mutations affect ovulation rate, which is the first limiting factor to litter size. Ovulation rate varies seasonally and can be increased by several nutritional or hormonal manipulations. Fertilization rate is affected by the ram, as well as by seasonal variations, hormonal manipulations of the ewe and methods of artificial insemination. In the late embryonic and fetal portions of pregnancy, partial losses occur more often than complete losses. Complete losses of embryos or fetuses after d 25 were greater in ewes that had lower progesterone at d 25 of gestation. Selection and management regimens should be developed to optimize prolificacy in relation to the geographic location, genetic background of the stock involved, input costs and overall environment of the operation.
Keywords: breeding value, ewe, litter size, prolificacy, ovulation

Introduction

Prolificacy (litter size) in sheep is dependent upon three major physiological functions: ovulation of ovarian follicles, fertilization of the ovulated oocytes, and survival of the resulting embryos through embryonic and fetal development and parturition. The latter two are dependent upon factors in both the ewe and the ram, but only the ewe will be considered in detail in this review. Attention will be given to genetic, nutritional, hormonal, and management factors that affect follicular development, selection of ovulatory follicles, survival of early embryos and losses during the late embryonic and fetal stages.

Ovulation Rate

Number of ovulations at a given estrus becomes the first limiting factor in prolificacy. It is usually assessed by counts of corpora lutea, but with the advent of observations by ultrasonography, can be estimated from the disappearance of large follicles from the ovaries during estrus. To assess the contribution of ovulation rate and examine how it might be optimized, several factors that affect it will be examined in some detail.

Because ovulation rate is determined by number of mature follicles at the time of the pre-ovulatory surge of luteinizing hormone (LH), it is largely dependent on the rates of follicular development and atresia. Therefore, we will briefly review the physiological control of follicular recruitment and selection. Interested readers are referred to an excellent recent review of the details of formation and development of follicles and factors affecting ovulation rate in ruminants (Scaramuzzi et al., 2011).

Ovarian follicles continuously grow to approximately 2 mm in diameter independent of gonadotropins. Beyond that point, follicles become not only more responsive to gonadotropins, but increasingly reliant upon gonadotropic support (Scaramuzzi et al., 2011). Small increases in follicle stimulating hormone (FSH) and threshold concentrations of LH recruit follicles into cohorts that are destined to ovulate or be lost. These gonadotropin-responsive follicles acquire the ability to secrete estrogen and become gonadotropin dependent. Thus, the number of follicles that continue to develop is dependent upon the amounts of FSH and LH available for this support, which likely leads to the observation of "waves" of follicular development from 3 to 5 mm in diameter. These larger follicles are dependent upon LH to support estrogen secretion, and are progressively lost if an LH surge does not occur. Concentrations of LH are regulated by concentrations of progesterone during the luteal phase, leading to frequent turnover of individual follicles and an average of about four waves per cycle in most breeds that have been studied. Thus, the maturation of follicles to a pre-ovulatory stage is dependent upon FSH for recruitment into

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final growth and upon LH for maintenance in a responsive state for ovulation.

A key contributing factor to ovulation rate is the period of time during follicular development in which ovulatory follicles grow to an ovulable size and remain responsive to an LH surge. It has been demonstrated in numerous studies that an increased number of ovulable follicles can occur by either retention of more follicles from the penultimate wave of development, recruitment of more follicles into the ultimate wave, or a combination of both. More follicles from the penultimate wave ovulate when luteal phase progesterone has been lower and secretion of pulses of LH has been more frequent during the previous luteal phase (Bartlewski et al., 1999; Knights et al., 2003; Devonish et al., 2009).

Genetic Control of Ovulation Rate

Obvious differences among breeds gave the initial clue that genetic factors influenced ovulation rate. It was assumed that prolificacy, a quantitative trait, would be controlled by multiple genes, but heritability estimates for numbers of lambs born were always low, around 10%. However, the trait was predictable. In flock records from which the influence of sub-fertile or infertile rams and the effect of ewe age had not been removed, year-to-year repeatability averaged 10.8% for number born and 12.9% for number raised (Inskeep et al., 1967). Lifetime performance of ewes that had averages of 1, 1.5, 2, or 2.5 or more corpora lutea at two observations in one breeding season were litter sizes of 1.23, 1.33, 1.47 and 1.56, respectively (Hulet and Foote, 1967).

The advent of a more in depth understanding followed the observation of greater ovulation rate in Merino ewes on the Booroola station in Australia by Helen Newton Turner (1978). We now know that the "Booroola gene" is on chromosome 6 and consists of a point mutation in the receptor 1B for bone morphogenetic protein-15 (BMP-15). The Booroola mutation increases secretion of FSH and is expressed in oocytes and granulosal cells in the follicle. A single copy increases ovulation rate by 1 or 2 oocytes and two copies by 3 to 10 oocytes above non-carriers. This gene affects ovulation rate by a number of mechanisms. There are more primordial and pre-antral follicles in their ovaries (Driancourt et al., 1985). Ovulable follicles are recruited over a longer period, undergo less selection, and remain in the window of opportunity to ovulate longer in Booroola Merino ewes than in control Merinos (Driancourt et al., 1985).

Other mutations, for which one copy can increase ovulation rate, but two copies yield no ovulations, have been identified in BMP-15 itself (on the X chromosome) or in growth differentiation factor-9 (GDF-9, on chromosome 5); both of these factors are expressed in the oocyte and are essential for ovulation. For detailed reviews, see McNatty et al. (2004) and Fabre et al. (2006). Recently, Silva et al. (2011) reported another polymorphism in GDF-9 in Brazilian Santa Ines sheep, in which homozygous ewes had an 82% increase in ovulation rate and a 58% increase in prolificacy.

Seasonal Regulation of Ovulation Rate

In breeds with strong seasonality, there are periods in the spring and summer when ovulation does not occur. Even within the breeding season, ovulation rate is lower early, peaks at mid-season and declines as the ewes approach anestrus (Hulet et al., 1974).

Nutritional Effects on Ovulation Rate

Nutritional "flushing" of ewes to increase ovulation rate has been practiced for many years, with a variety of different approaches, from use of grain feeding or turning into aftermath meadows, to increasing nutrition by either method after a period of deliberate limited pasture or range quality. Early work led to the concept that there were effects of both static (longer term nutritional status as reflected by body condition) and dynamic (temporary increases) nutritional signals on ovulation rate (Coop, 1966). Attempts to identify the mechanism of nutritional effects began in the 1960s with studies of gonadotropin content of the anterior pituitary and follicular development in the ovary (e. g., Bellows et al., 1963). Memon et al. (1969) found that energy had a greater effect than protein when either or both were increased above a maintenance regimen. In a recent review, Scaramuzzi et al. (2011) concluded that flushing effects occur mainly at the ovary, with the dynamic (short-term) response signaled mainly by glucose and insulin, and body condition acting primarily through growth hormone, insulin like growth factor (IGF), and the adipose tissue hormone, leptin. These signals regulate functions in the oocyte and follicle, and whether the oocyte is ovulated or lost through follicular atresia is determined in part by its actions on the follicle.

In early work, it appeared that nutritional flushing needed to continue for at least the length of an estrous cycle to be effective. A more rapid action was observed in ewes with a synchronized follicular wave (Viñoles et al., 2010). Supplementation for just six days, beginning on the day of wave emergence, increased emergence of a second follicular wave on the fourth day, and tended to increase the rate of ovulation of follicles in that wave in ewes in high (1.7 vs 1.3), but not low (1.1 vs 1.2) body condition. In three of seven supplemented high condition ewes, ovulations occurred from both the first and second waves, despite the short interval for development of the second wave.

Hormonal Factors in Ovulation Rate

As stated above, lower progesterone encourages increased pulse frequency of LH and increased ovulation rates, which may be reflected in increased prolificacy, if prolificacy in control ewes is relatively low in relation to their genetic potential. Knights et al. (2001a, b) observed no significant benefit of treatment with FSH in anestrous ewes that received progesterone via controlled internal drug releasing (CIDR) inserts for 12 or 5 d before ram introduction. Similarly, melatonin implants increased prolificacy in groups of ewes in which controls had fewer lambs born per ewe, but not when litter size was already

approaching twinning (Lopez-Sebastian and Inskeep, 1991).

Hormonal treatments with gonadotropin releasing hormone (GnRH), LH, equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG) or combinations such as P.G. 600, which contains both eCG and hCG, have been used in numerous trials as a means to increase ovulation rate and prolificacy. Effective approaches to superovulation for embryo transfer have been reported (see review by Gonzales-Bulnes et al., 2004). Results in out-of-season breeding or with regimens for synchronization of estrus have varied with dosage, timing, and treatments used in combination with the gonadotropin, but effective increases in lambs born per ewe lambing have been rare and pregnancy rates have sometimes been compromised. Because most of the important variables are discrete, rather than continuous, results based upon small numbers of ewes cannot be considered reliable for predicting results, especially when breed, season of the year, body condition, ewe:ram ratio, and preparation of rams and ewes have varied. Safranski et al. (1992) treated anestrous ewes with the orally-active progestogen, melengestrol acetate (MGA) and P.G. 600 (200 I.U. eCG and 100 I.U. hCG) at the last feeding of MGA. They found more ewes ovulating and a greater ovulation rate with P.G. 600 than with MGA alone, but no change in lambs born per ewe lambing or per ewe exposed. In recent trials in West Virginia, treatment of non-lactating anestrous ewes with a slightly greater dose of P.G. 600 (240 I.U. eCG and 120 I.U. hCG) at removal of a 5-d CIDR treatment had no value, but treatment with that dosage on the day before removal of the CIDR showed promise in lambs born per ewe treated (K. D'Souza and M. Knights, unpublished).

Another treatment that has been tested and used extensively is immunization against ovarian hormones, including estradiol, androstenedione, and inhibin. The most successful in increasing ovulation rate was immunization against androstenedione, the concept being that this would reduce ovarian secretion of estrogen (because androstenedione is a precursor of estrone and estradiol) and thus negative feedback on FSH (Scaramuzzi et al., 1977; Gibb et al., 1982; see review by Smith, 1985). This treatment has been applied extensively in Romney ewes in New Zealand.

Fertilization Rate

In the ewe, fertilization rate has been studied in a variety of situations, most often with few animals, and in relation to manipulations such as superovulation or laparoscopic insemination. Whether fertilization or failure was complete or partial within a ewe often has not been reported, so useful assessments of factors affecting normal fertilization rates are rare. One interesting observation was that 81 to 95% of ova recovered at 3 d post mating were fertilized in breeding season ewes kept at 70°F compared to 56 to 60% in ewes kept at 90°F since 5 d before estrus (Alliston and Ulberg, 1961). It should also be kept in mind that ram fertility can have important effects on fertilization rate. For example, Hulet et al. (1956) attributed high rates of fertilization failure (34 to 64% unfertilized) early in the breeding season to defective ova in 3 to 17% of cases and poor sperm from rams in 31 to 48%, using data collected in two consecutive years. Thus the importance of breeding soundness exams and libido of rams cannot be overemphasized.

Factors That Influence Embryonic and Fetal Survival

Successful pregnancy requires continued secretion of progesterone by the corpus luteum for approximately 50 d (Casida and Warwick, 1945), after which the placental secretion of progesterone is adequate. Maternal recognition of pregnancy involves secretion of interferon tau by the conceptus and increased secretion of the E prostaglandins (Silvia et al., 1984; Bazer et al., 2008; Hansen et al., 2010), to maintain the corpus luteum in the face of increased $PGF_2\alpha$, which is probably also necessary for attachment of the conceptus. Total failure of these processes, in combination with fertilization failure and very early embryonic loss, varies. However, loss during maternal recognition is likely rare, because extended cycles are rarely observed.

Hulet et al. (1956) estimated early embryonic death at 29% (20% before d 18 and 9% from d 18 to term) in ewes mated early in the breeding season and 10 % in ewes mated later. After breeding during summer anestrus, 28% of mated ewes were not pregnant at d 25 (Dixon et al., 2007). Open ewes could be attributed to failures of ovulation or fertilization or early embryonic death. In the study by Knights et al. (2003), number of corpora lutea at ultrasonography on d 10 to 14 was 2.1 and an average of 1.9 embryos was present in pregnant ewes at d 25. After d 25, 21% of embryos present at that time were lost, 3 to 4% of embryos or fetuses during each 20 to 25 d of the remainder of pregnancy. Partial losses of pregnancy were more frequent than complete losses of either single or multiple embryos or fetuses (Dixon et al., 2007). However, subsequent complete losses were associated with low progesterone at d 25.

Placental angiogenesis, adequate attachments of fetal cotyledons to maternal caruncles, and blood flow to the utero-ovarian complex have all been suggested as limiting factors that might contribute to partial losses. Based upon earlier data in pigs and rats, Luther et al. (2008) treated ewes on d 0 to 15 after mating with 27 mg arginine once daily to stimulate nitric oxide secretion and thereby increase blood flow to the ovaries and uterus. They found no effect on pregnancy rate, but 0.3 more embryos and 0.4 fewer corpora lutea not represented by embryos at d 25 in arginine-treated ewes than in ewes receiving saline.

Losses during pregnancy varied with breed group of the ewe, being greater in black-faced ewes than in white- or mottled-faced ewes in the study by Dixon et al. (2007). However, in another study, losses were greater in white-faced ewes, especially dairy sheep breeds (Holler, 2011). In that study, losses differed with ram breed groups and individual rams as well as with genetic background of the ewe. Losses occurred during pregnancy regardless of whether ewes were mated at synchronized estrus, in or out of season, or at natural estrus in season. Ovulation rate

increased with age, and perhaps surprisingly, losses also increased with age of ewe. That result may simply reflect the fact that losses increased with ovulation rate, as shown in numerous studies reviewed by Devonish et al. (2009). The latter authors studied the effects of circulating concentration of progesterone on ovulation rate, embryonic and fetal losses, and litter size in prolific Barbados Blackbelly ewes. As mentioned earlier, ovulation rate increased as concentration of progesterone decreased, but the number of corpora lutea not represented by lambs born also increased, so that litter size remained constant, regardless of concentration of progesterone. Thus average litter size, in ewes that become pregnant and retain pregnancy, is usually less than ovulation rate.

Conclusion

Ovulation rate sets the upper limit on prolificacy and is influenced by a number of factors, including time of year, genetic (breed) differences, and nutritional status of the ewe. Fertilization rate and embryonic and fetal loss then decrease the maximum prolificacy rate. Fertilization rate is usually relatively high except during anestrus or the transition season, so most losses are due to embryonic or fetal death. The reproductive fitness of the ram has a major impact on these factors that decrease prolificacy and should thus be taken into account in any attempts to maximize lamb production.

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2013 Sheep Symposium[1]
MATERNAL ENVIRONMENT IMPACTS FETAL AND OFFSPRING OUTCOMES IN SHEEP

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ABSTRACT: Developmental programming, defined as a stimulus or insult during a critical period in conceptus development establishing a permanent physiological response, is of growing concern in livestock production. Factors that could impact conceptus development include: genetics, maternal age, litter size, maternal activity level, or nutrient availability. Decreased nutrient availability during early pregnancy may hinder placental development, ultimately restricting fetal growth during later stages of gestation. Poor maternal nutrition during later stages of pregnancy can equate to reduced birth weights due to inadequate placental nutrient supply and concomitant transfer to the fetus. Ultimately, maternal nutritional intake can impact uteroplacental blood flow, and thus nutrient delivery to the offspring. Low birth weights not only impact neonatal morbidity and mortality, but more recently, have been linked to poor growth performance and carcass quality. Furthermore, maternal diet has also been shown to impact reproductive performance of the offspring. Elucidating the consequences of inadequate maternal intake on the continual plasticity of placental function will allow us to determine the proper timing and duration for intervention in our livestock species.
Keywords: conceptus, nutrient restriction, placenta, pregnancy, uterus

Introduction

Sheep producers are interested in utilizing nutrients in the most efficient way to optimize growth. Often, one tends to focus on the growth that an animal achieves after birth; however, sheep can spend 25 to 50% of their life (i.e. from conception to consumption) within the uterus, being nourished solely by the placenta. Therefore, it is especially relevant to understand the impacts of the maternal environment on placental growth and development as this directly impacts fetal growth. The maternal system can be influenced by many different extrinsic factors, including nutritional status, which ultimately can program nutrient partitioning, and consequently growth, development, and function of the major fetal organ systems (Wallace, 1948; Wallace et al., 1999; Godfrey and Barker, 2000; Wu et al., 2006). Fetal organogenesis occurs simultaneously with placental development. As the growth trajectories for these tissues vary, each tissue is susceptible to suboptimal conditions (i.e. maternal undernutrition) at different periods. Depending upon the production objectives, the organ systems of greatest value may be those that contribute to carcass traits (i.e. muscle and fat development) as well as reproductive performance of replacement females.

Impacts of Maternal Nutrition on Skeletal Muscle Development

As skeletal muscle during fetal development has a lower priority in nutrient partitioning compared to the brain and heart, it is particularly vulnerable to nutrient deficiency (Bauman et al., 1982; Close and Pettigrew, 1990). Moreover, as there is no net increase in the number of muscle fibers after birth, the fetal period is critical for skeletal muscle development (Glore and Layman, 1983; Greenwood et al., 2000; Nissen et al., 2003). Maternal nutrient restriction can significantly reduce the number of both muscle fibers and nuclei in the offspring (Bedi et al., 1982; Wilson et al., 1988; Ward and Strickland, 1991). Nutrient restriction in ewes from early to mid-gestation results in a reduction of fetal skeletal muscle fibers, which may be related to a down-regulation of mammalian target of rapamycin (mTOR) signaling (Zhu et al., 2006). The mTOR pathway is believed to mediate nutrient signals such as amino acid sufficiency (Fumagalli and Thomas, 2000; Gringras et al., 2001) and provides a link between nutritional levels and skeletal muscle development (Erbay et al., 2003).

In our laboratory, we have obtained slaughter data collected from lambs born to ewes that have been globally restricted or overfed by 40% compared with controls (i.e. 100% NRC recommendations). While our lambs had similar hot carcass weight, individual dissected muscle weights (i.e. semimembranosus and psoas) were reduced in lambs from overfed ewes compared to control-fed ewes (Harris et al., 2009). When we compared lamb carcass measurements from ewes fed isocaloric diets but differing levels of protein during the last third of pregnancy, we did not observe any differences (Van Emon, 2013). In steers born from cows that received a protein supplement during

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the last third of pregnancy, hot carcass weight was also similar to non-supplemented controls (Larson et al., 2009). However, marbling score and those grading Choice were increased in those steers born to protein supplemented cows during late gestation (Larson et al., 2009). The differences between our sheep protein studies and cattle research may be species variation. It could, however, be influenced by total caloric intake as well. In our sheep study, ewes had similar caloric intake throughout the study, whereas in the beef cattle studies, the additional supplement, although high in protein, also just added more calories to the diet.

Impacts of Maternal Nutrition on Reproductive Tract Development

During periods of maternal nutrient restriction, offspring reproductive tissues would also be a low priority during fetal development. Recent evidence indicates that reproductive performance of female offspring from nutrient compromised dams can be reduced. Heifers born from multiparous cows given a protein supplement during the last third of pregnancy had an increased pregnancy rate compared to heifers from non-supplemented dams (Martin et al., 2007). Fewer heifers from non-supplemented dams attained puberty before the first breeding season compared with heifers from supplemented cows in a subsequent study (Funston et al., 2010). Additionally, in rats where dams were protein restricted during pregnancy, female pups had a delay to vaginal opening (i.e. puberty) and time to first estrus compared to control dams (Guzman et al., 2006).

Our laboratory has also shown that maternal diet can impact the fetal reproductive tissues. Fetal ovaries from ewes experiencing a 40% nutrient restriction had a decreased cellular proliferation rate in primordial follicles compared to ovaries from fetuses of adequately fed ewes (Grazul-Bilska et al., 2009). This decreased proliferation in the primordial follicles follicle may impact future follicular activity, fertility, and reproductive longevity of the female offspring. Unfortunately, these data do not provide information regarding the reproductive success of these offspring.

Recently, we have demonstrated that if ewes are fed isocaloric diets with varying levels of protein during late gestation, ewe lamb reproductive performance can be altered. Ewes fed diets that are similar in total energy, but reduced in protein during late gestation, have ewe lambs that give birth later in their lambing season. This could be due to later attainment of pregnancy or an increased embryonic loss occurred early in the breeding season. Just as interesting, if the percentage of the diet was enhanced in protein content, there was no advantage in lambing criteria of the resultant ewe lambs. In fact, our laboratory has noted that lamb birth weights in ewe lambs born from dams fed excess protein have offspring with a lower birth weight compared to control fed ewes (Van Emon, 2013).

Can We Impact Postnatal Performance by Enhancing Placental Function?

Regardless of the experimental approach employed to study intrauterine growth restriction, uteroplacental blood flow is reduced (Reynolds et al., 2005). In approaches where the pregnant ewe is undernourished, nutrient availability in maternal plasma is reduced and therefore uptake by the gravid uterus is limited. We have recently demonstrated that when ewes are nutrient restricted during mid to late gestation, umbilical blood flow is reduced by ~30% compared to controls (Lemley et al., 2012). However, if pregnant ewes are supplemented with melatonin during the second half of gestation, umbilical blood flows can increase to levels that are experienced by adequately fed ewes (Lemley et al., 2012). Further investigations are underway to associate how placental function is linked to post-natal performance.

Conclusion

The continual desire to enhance management methods to produce healthy lambs has led to increased research in the area of developmental programming. By understanding how the maternal system can, or cannot, adapt to differing stressors during normal pregnancies, we can develop interventions or therapeutics to augment placental development and enhance nutrient delivery in order to produce optimally developed offspring.

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2013 Sheep Symposium[1]
LET'S GROW: ASI INITIATIVES FOR INDUSTRY GROWTH

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ABSTRACT: The American Sheep Industry Association (ASI) began an initiative in 2011, called *Let's Grow*, to grow the national flock in order to provide a strategy to strengthen the industry's infrastructure for long-term sustainability. This article will discuss the promotion of the program, resources developed, programs that were supplied funding, and the 2013 Scope of Work.
Keywords: promotion, let's grow initiative, sheep industry, sustainability

Promotion

The focus of the work in 2011 on the *Let's Grow* initiative was to promote its launch, which included the development of a website (www.growourflock.org) marketing materials, and media promotion. The site includes a resources section that hosts downloadable management practices (written by a group of dedicated sheep extension specialists) and links to various webinars that have been hosted during the past year, among other items.

Immediately following the launch of the campaign at the 2011 sheep industry convention, ASI leaders promoted the *Let's Grow* initiative on RFD-TV Live. A variety of sheep industry issues were covered but the highlights included the goals of the initiative, a recap of the lamb and wool markets, the benefits and ease of adding sheep to already existing agriculture enterprises, resources for new producers looking to get into the industry and the array of industry information that can be found at www.sheepusa.org. In addition, the audience viewed a series of sheep producer documentaries depicting their success in the industry and portraying the various methods in which to raise sheep. Those documentaries can be viewed at http://www.sheepusa.org/ASI_on_RFD-TV. The hour-long show concluded with live calls from the audience, which ranged in topics from Livestock Risk Protection for Lamb, how the industry is promoting lamb, and sheep stocking rates.

The media promotion prong consisted of media events held in Iowa, Minnesota, Ohio, Indiana, Tennessee and California. Representatives from ASI provided an overview of the demand and supply situation; steps the industry is taking to help grow flock size; how individual producers can expand their flocks; available markets; and how young producers, individuals interested in a rural lifestyle, and how producers of other livestock can get involved in the industry. Local producers were also available to share their stories and how they plan to help grow the industry. After the initial launch of *Let's Grow*, the work in 2012 focused on the development of a mentor program and increasing sheep production efficiency.

Resources Developed

ASI developed a sheep production tool kit targeting emerging producers, which includes a host of information to teach new producers about the production of safe and wholesome food and fiber, the well-being of sheep, sheep diseases, ration balancing software and educational opportunities and courses. Also included in the package is a complimentary digital version of ASI's Sheep Production Handbook. Mentoring guidelines were developed to assist state sheep associations in the development of their individualized programs and a webinar was hosted giving even further insight to the mentoring relationship. In addition, under the *Let's Grow* initiative, ASI provided states with a $1,000 grant to assist in the development of their individualized program to help emerging producers succeed in the industry. The focus of increased efficiency in sheep production came with the development of additional management practices posted to the *Let's Grow* site in addition to the hosting of production focused webinars for the industry.

Funded Programs

A major funding program for this initiative in 2012 was the SheepSD project, a South Dakota State University Extension program designed to help sheep producers enter and expand into the sheep industry. This 3-yr program provides a curriculum to help equip new producers with the tools to make wise management decisions, in turn contributing to ongoing sheep production, land stewardship, and rural community viability.

2013 Scope of Work

The scope of work in 2013 will continue with the focus on production efficiencies including the development of additional management practices, continued distribution of

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tool kits for new sheep producers, and again offering a $1,000 grant to member state sheep associations for mentoring activities and continued support of the SheepSD program.

Another launch that will take place this year is the addition of a Sheep Community of Practice to the eXtension system, which can be found at www.extension.org/sheep. Producers will be able to access research-based articles, take online courses, participate in real-time webinars, and utilize an "Ask an Expert" function. Once the site is fully populated, it will contain research-based information on seven key areas: feed and nutrition, reproduction and breeding, management practices, genetic selection, health and veterinary care, grazing and pastures and wool.

Conclusion

ASI believes the initiative has been successful, even given the circumstances of the drought, fires, and market price softening. According to an analysis of the sheep industry that was made by the U.S. Department of Agriculture's, Economic Research Service in its Livestock, Dairy and Poultry Outlook, "In 2012, sheep inventory registered a smaller decline than in the previous two years. Despite the drought conditions in most of the sheep-producing areas, the 'Grow Our Flock' program by the sheep industry appeared to slow the decline. The National Agriculture Statistics Service's Sheep and Goats report estimated the inventory of all sheep and lamb in the United States on Jan. 1, 2013, as 5.34 million head, down 1 percent and a 30,000-head decline from 2012. Breeding sheep inventory decreased to 3.98 million head on Jan. 1, 2013, down 1 percent from 4.0 million head on Jan. 1, 2012. A 2-percent decline was seen in the 2012 lamb crop and is expected to result in further lamb production declines during 2013, with production at around 152 million pounds. As a result, slaughter lamb prices, which declined significantly in 2012, are expected to show strength in 2013..." (USDA, 2012).

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2013 Sheep Symposium[1]
ALTERNATIVE FEEDS: A SOLUTION FOR A TEMPORARY CRISIS OR A PERMANENT PROBLEM?

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ABSTRACT: Feed costs represent a significant portion of the total cost of livestock production. Historically, when traditional feed costs are inflated, alternative feed ingredients are more thoroughly researched, discussed, and eventually used in livestock diets. As the price of feed ingredients return to normal, use of alternative feeds quickly subsides. However, recent factors including drought, rising fuel costs, and competition for biofuel feed resources have caused an unprecedented rise in feed costs. These factors, along with current issues such as economic stagnation, greater emphasis on enhancing natural resources, and increased environmental and livestock production regulations, suggest that a temporary crisis may be developing into a permanent problem. Numerous alternative human food and crop residues, (e.g. bread, candy, cotton gin trash) have been researched and used to help stabilize inflated feed costs, but they are not always available, have variable nutritional characteristics, and can be difficult to handle. In contrast, an alternative feed does exist, which is abundantly available throughout North America, requires no inputs such as fertilizer, irrigation, pesticides, or herbicides, and is highly resilient to drought and market volatility: woody plants. Therefore, the process of converting woody plants to feed should be revived by making it more efficient, enhancing the nutritional value of the final products, and documenting benefits to the animal, natural resources, and rural economies. Currently, no other program is available that can economically, or even theoretically, reduce brush encroachment while concurrently producing a livestock feed ingredient that is cost competitive to traditional roughages.
Keywords: alternative feeds, feed cost, livestock production, juniper, roughage

Temporary Crisis or Permanent Problem?

The change in feed costs over the past decade is alarming and has proven to be unsustainable for many livestock production sectors. Rising feed costs (Figure 1) can be attributed to many factors, such as rising fuel costs and federal mandates of feed resources being diverted to biofuels production (EIA, 2012; Wise, 2012). These costs are exacerbated by matters such as inflation and drought-induced feed shortages. One notable example is the current price of cottonseed hulls (CSH), which provide little nutritive value and function mainly as a roughage ingredient to maintain rumen function (Table 1). As of February 15, 2013, Texas markets priced CSH at $145/dry ton, not including average freight of $3/loaded mile (Hansen-Mueller Inc., McKinney, TX). During the 2011 drought, CSH sold for approximately $350/ton in some markets until they were no longer available in Lubbock (Feedstuffs Magazine, October 5, 2011).

Currently, roughage ingredients of similar nutritional quality to CSH (Table 1) are difficult to economically justify in livestock diets when priced above $130/ton. The question is, how many times within the next 5 yr will CSH be priced below $130? Between June, 2010 and February, 2013, CSH were priced less than $130/dry ton only 85 out of 177 reports; only 3 times between January, 2012 and February 2013 (Texas markets; Hansen-Mueller Inc., McKinney). Higher quality roughage ingredients such as alfalfa hay have an even more discouraging price history for the livestock feeder.

During times of feed shortages and elevated feed costs, livestock producers are more predisposed to utilize alternative feeds, even if those feeds have not been thoroughly researched or analyzed for nutrients. As the cost of traditional feed ingredients returns to normal, use of alternative feeds subsides. For instance, high feed prices during 1918 to 1919 and during the 1930's, resulted in greater research and use of sawdust and ground aspen trees in livestock diets (Davey, 1977; NRC, 1983); however, use during both periods halted as the price of traditional ingredients returned to normal. Even though woody residues have not been generally recognized as competitive feed alternatives under normal economic conditions (Scott et al., 1969), it is notable that *Populus* trees were approved as an Association of American Feed Control Officials feed ingredient in 1980 (AAFCO, 2011).

We predict that livestock producers will need to increase use of precision diet formulation to maximize gain-to-feed efficiency and increase feed storage capacity to help stabilize feed price fluctuations. In addition, greater transportation and feed costs will encourage onsite confined feeding operations and grazing systems that utilize local feed resources to reduce time spent in the feedyard. These predictions are partially based on the fact that the regulatory burden on large concentrated animal feeding operations is rapidly increasing. In some areas, this will lead to a greater demand for brush control to enhance forage production.

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We also predict that membership in livestock cooperatives will become more important, to share financial burdens, equipment, labor, and expertise. These predictions justify the establishment of a national *Wood-to-Feed Program* that will remain economically and environmentally sustainable even if traditional feed ingredients become "reasonable" once again.

Alternative Feed Examples and Considerations

The number of alternative feed ingredients that have been used in livestock diets is extensive and cannot be thoroughly discussed in this paper; however, the following references provide abbreviated summaries: (NRC, 1983; Lardy and Anderson, 2003; Blache et al., 2008). Before potential feed ingredients can be commercially fed to livestock, feeds must first be approved through FDA or AAFCO procedures to ensure the feeds are safe for the animal and do not result in residual compounds in milk or meat that affect human health. Furthermore, certain issues need to be considered before using any feed, especially alternative feeds that have not been extensively researched. For example, depending on concentration certain secondary plant compounds, i.e. condensed tannins (CT) and terpenoids, can either reduce animal performance (Barry and Forss, 1983; Pritz et al., 1997; Blache et al., 2008) or increase animal performance (Waghorn et al., 1987; Min et al., 2001; Ramirez-Restrepo and Barry, 2005).

Diets containing greater than 5% mesquite leaves can reduce intake and BW gain in sheep (Baptista and Launchbaugh, 2001), due to compounds such as flavonoids, phenolics, and alkaloids (Cates and Rhoades, 1977; Solbrig et al., 1977; Lyon et al., 1988). Other compounds to consider are phytoestrogens and certain minerals such as Se, as previously reviewed (NRC, 1983; Blache et al., 2008). Thus, analyzing each ingredient for chemical composition and purity is especially important in alternative feeds. Two other important considerations include the need for specialized facilities and equipment to store, move, and process low-density feeds and the use of pre-treatment technologies (e.g. air- and oven-drying, ensiling, and pelleting) that can reduce concentrations and bioactivity of secondary plant compounds. Each individual buyer will need to use research, experience, or both, to determine if the ingredient is worth purchasing.

Development of the Texas AgriLife *Wood-to-Feed Program*

Goats will consume juniper leaves while grazing (Malachek and Leinweber, 1972); thus, our first experiment centered on feeding lambs mixed diets that contained redberry juniper leaves. Because blueberry juniper was more readily browsed than redberry juniper (Riddle et al., 1996) and goats consumed more juniper than sheep (Straka, 1993), we hypothesized that our results would be even more relevant to goats and blueberry juniper. In this experiment, replacing 50% of the CSH with redberry juniper leaves increased animal performance in lambs, compared to diets containing CSH or juniper leaves as the sole roughage source (Whitney and Muir, 2010). These results led to a study in which mixed diets containing ground juniper leaves and small stems were fed to lambs. The juniper successfully replaced all of the oat hay in diets containing 40% DDGS (T. R. Whitney, unpublished data). Additional studies showed that redberry juniper-based diets can reduce *Haemonchus contortus* infection (Whitney et al., 2011; T. R. Whitney, unpublished data) and that other *Juniperus* spp. have similar nutritional characteristics as compared to redberry juniper (Table 1).

A further review of the literature revealed a wealth of information related to successfully incorporating woody material into livestock diets (e.g. Sherrard and Blanco, 1921; Archibald, 1926; Hvidsten, 1940; Nehring and Schutte, 1950; Marion et al., 1957; Parker, 1982; NRC, 1983). These reports, along with numerous others, demonstrate a potential to reduce woody plant encroachment, while synergistically developing a low-cost livestock feed ingredient. For example, Marion (1957) reported that steers fed mixed diets containing 50% ground mesquite wood performed similar to steers fed 50% CSH and that the mesquite meal cost 44% less than CSH. So, why did this process not develop into a permanent production practice? Many suggest that the low cost of traditional roughage sources did not justify the additional labor and equipment costs needed to convert standing trees into feed. However, machinery and techniques available today are much more capable and efficient in converting large quantities of standing trees into quality hammer-milled feed products. Also, brush encroachment has become the center of attention for natural resource, livestock, and wildlife management and soil and water conservation, and the current price of roughage feed ingredients justifies an integrated program that converts woody plants into feed.

The Texas AgriLife *Wood-to-Feed Program* has been developed from almost a century of documented research efforts, advanced technology, entrepreneurship, and foresight of our predecessors. The primary goal is to increase the value of encroaching woody plant species to reduce harvesting costs, while synergistically increasing grass production and ecosystem health, and reducing livestock feed costs. Multiple scientists and industry partners with complementary backgrounds and specialties are collaborating to rapidly increase adoption rate of this proven practice.

Implications

Rising livestock feed costs will necessitate changes within all livestock industries. Production practices will shift as producers address feed ingredient shortages. Energy, economic, and regulatory challenges will accelerate adoption of feeding alternative ingredients in livestock diets. The feasibility of any alternative feed depends on cost and availability. While the cattle industry is an excellent outlet for woody feed ingredients, small ruminants stand to benefit from utilizing ground woody plants perhaps even more than cattle, in part due to their suitability to landscapes where woody plants dominate. In certain circumstances, woody plants are an on-site feed resource on many sheep ranches throughout the U.S. Numerous

benefits to rangelands, the livestock industry, and local economies will be recognized when large amounts of brush are harvested for livestock feed. Producers should be ready if the current price of feed transitions into a permanent problem.

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Table 1. Chemical composition (%, DM basis) and 48-h IVDMD of traditional and alternative feed ingredients[1]

	Nutrient[2]					
Item	CP	NDF	ADF	Ash	CT	IVDMD
Alfalfa, mid-bloom hay	17	47	36	9	-	71
Coastal bermudagrass hay	10	73	36	6	-	56
Sorghum-sudangrass hay	8	67	43	10	0.9	50
Cottonseed hulls	6.6	79	69	2.7	5.6	21
Redberry juniper, leaves	7.1	37.7	31.2	5.3	5.5	67
Redberry juniper, whole tree	3.6	66	56	4.2	4.7	29
One-seed juniper, whole tree	3.6	64	53	4.4	2.7	32
Eastern red cedar, whole tree	3.7	68	58	3.5	5.6	29

[1]NRC, 1983; NRC, 2007;Whitney, T. R., and J. P. Muir. 2010; W. C. Stewart and T. R. Whitney, unpublished data.

[2]CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; CT = condensed tannins; IVDMD = invitro dry matter digestibility.

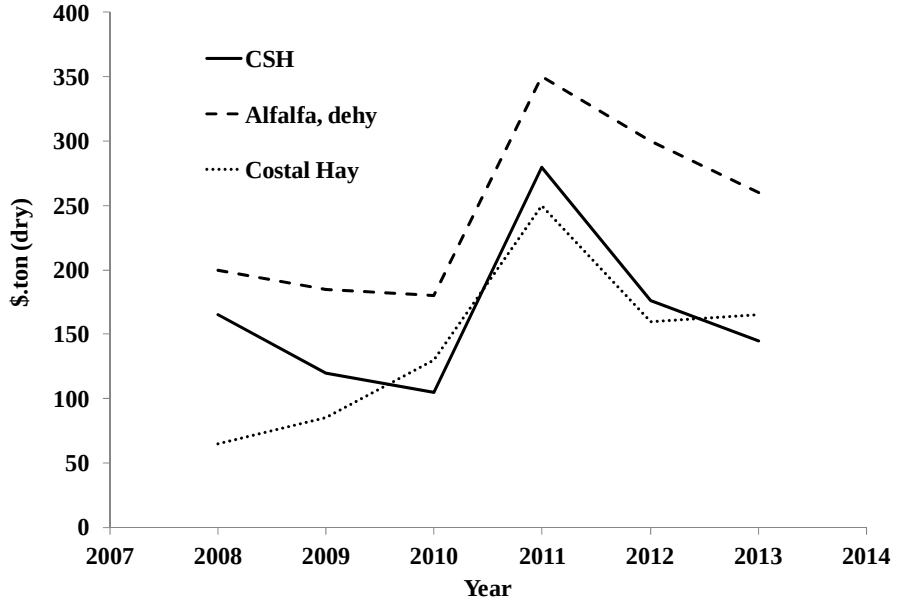

Figure 1. Market prices (at source and not delivered) for traditional roughage ingredients: cottonseed hulls (CSH; Hansen-Mueller Trading, McKinney, TX); dehydrated alfalfa (Alfalfa, dehy), and round bales of coastal bermudagrass hay (Costal Hay; USDA-AMS, 2008-2013).

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2013 Sheep Symposium[1]
ALTERNATIVE GRAZING STRATEGIES FOR INDUSTRY DIVERSIFICATION AND RANGELAND IMPROVEMENT

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ABSTRACT: Carefully managed sheep grazing can be an important tool to manage the health of rangeland ecosystems, while maintaining production on these lands. Grazing lands are increasingly lost to development, cultivation, invasive species, and wildlife habitat preservation, while livestock production must increase to meet rising demands for food from a growing population. Rangeland managers and livestock producers must explore ways to intensify livestock production on remaining rangelands and develop alternative grazing strategies to benefit production and improve land health. Many opportunities exist for producers to participate in restoration grazing strategies that increase producer access to forage and grazing lands, while helping to improve the efficacy and longevity of other vegetation management tools. Sheep grazing has been successfully integrated with herbicides, mechanical treatments, prescribed burning, and insect biological control agents to improve the success of vegetation management. Targeted sheep grazing, combined with insect biological control agents, that attacks plants both above and below ground has strong potential to restore large expanses of rangeland, while still garnering an economic return from these lands. Restoration projects initiated with an integrated plan that includes grazing management from start to finish are more likely to be successful ecologically and economically. It is important to have well-defined goals for the final vegetative composition as well as the economic investment of each project.
Key words: integrated management, invasive weeds, restoration, sheep, targeted grazing

Introduction

Rangelands account for more than 40% of the world's land area and are classified as the most degraded land type in the world (Lund, 2007). Furthermore, access to grazing lands is being lost to development, cultivation, invasive species, and wildlife habitat preservation, while livestock production must increase to meet rising demands for food to support worldwide population expansion (FAO, 2003).

Rangeland managers and livestock producers are exploring ways to intensify livestock production on remaining rangelands, while simultaneously developing alternative grazing strategies to improve land health (Estell et al., 2012). Papanastasis (2009) advocates that rangeland degradation is the result of mismanagement of livestock, rather than their mere presence, and asserts that the solution does not lie in exclusion of livestock grazing from rangeland restoration projects, but in adapting grazing management to help restore degraded rangeland. Numerous opportunities are available for sheep producers to stretch their home forage base, while performing grazing for rangeland restoration objectives. While these opportunities are not without potential risk to production efficiency, their continued development may enhance the sheep industry over the long-term by improving rangeland health and sustainability of grazing operations.

Sheep have proven to be valuable tools for restoration and vegetation management in rangelands, cropland, and plantation settings; and can target a variety of annual grasses, broadleaf weeds, and shrubs (Launchbaugh and Walker, 2006). However, vegetation change through grazing alone is a slow process. For example, it often requires 4 or more years of sheep grazing before a decline in perennial weeds is documented and weeds may return to near pre-grazing levels within a few years of sheep removal (L. Surber and R.W. Kott, Montana State Univ., Bozeman, MT, personal communication). Consequently, the strategy of controlling herbaceous, invasive weeds should focus on integrated management, which involves the combined use of two or more management tools to achieve a desired objective on the landscape. Integration generally involves using one tool to alter the structure or composition of the existing plant community to improve the success of the subsequent tool. Through research identifying effects that grazing can have on the structure, age class, and reproductive output of the plant community, sheep grazing can mold the plant community into something more receptive to other treatments, rather than trying to suppress a target plant with grazing alone.

Sheep grazing can impact the plant community in several ways, most commonly by reducing the biomass and cover of herbaceous vegetation. Reduction in biomass can affect fire behavior (Nader et al., 2007), while changes in cover and litter can affect plant species recruitment (Oesterheld et al., 1990). Grazing also impacts the species composition of the landscape. Sheep generally prefer broadleaf forbs to grasses and these natural preferences can

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be used to gradually shift the composition of a plant community to a more balanced or desired state (Gibson et al., 1987; Krahulec et al., 2001). Selective grazing by sheep can also alter the age distribution of plant species. Olson et al. (1997) reported that sheep prefer the young, tender rosettes of spotted knapweed (*Centaurea stoebe*) as opposed to older plants with more structural fiber.

Sheep grazing can effectively control the seed production of many invasive plants such as spotted knapweed (Olson et al., 1997), leafy spurge (*Euphorbia esula*; Lym, 1988), and sulfur cinquefoil (*Potentilla recta*; Frost et al., 2011). Specifically, Olson et al. (1997) demonstrated that 3 yr of continuous grazing by sheep reduced the seedbank of spotted knapweed by 75% compared with controls. Having a large seed bank is important, even for perennial weeds, for weedy species to maintain dominance of a site (Seastedt et al., 2007). Grazing when seeds are formed but not viable reduced viable seed rain of sulfur cinquefoil by 65 to 97% (Frost et al., 2011). The ability of sheep to manage seed production is a valuable tool, particularly for plants that have no potential biological control agents. For example, agencies in Idaho and Montana are exploring the use of sheep or goats as a means to suppress seed production of rush skeletonweed, an invasive plant that reproduces by massive amounts of windborne seed, to prevent expansion from remote areas of Idaho into Montana (K. Goodwin, Montana State University, Bozeman, MT, personal communication).

Integration for Restoration

Sheep grazing has been successfully integrated with herbicides, mechanical treatments, prescribed burning, and insect biological control agents to improve the success of vegetation management.

Grazing and Herbicides

Grazing has been successfully integrated with herbicides to either stress plants before herbicide application or to control residual plants following herbicide application. Researchers in North Dakota used intense goat grazing during the growing season to remove above-ground foliage and stress the root system of leafy spurge. They followed with a fall application of herbicide and determined that control lasted longer than either grazing or herbicide applied alone (Lym et al., 1997). Sheley et al. (2004) demonstrated that successful control of spotted knapweed could be obtained when sheep grazing was implemented following treatment of spotted knapweed with the less expensive, low-residual herbicide, 2-4,D. The 2-4,D controlled the mature knapweed plants, but new seedlings emerged the following year. These new seedlings were readily consumed by sheep that prefer young, vegetative knapweed over mature, fibrous plants (Olson et al., 1997).

Grazing and Prescribed Fire

There is great potential to integrate grazing and prescribed fire to reduce the incidence of wildfire and control invasive annual grasses. Sheep grazing may be most useful in moderating the fuel load on large areas of rangeland with the intent of reducing flame height and altering fire behavior by changing the fuel bed depth, fuel loading, and percent cover (Nader et al., 2007). While grazing can be used to reduce biomass of perennial grasses, the greatest potential for restoration lies in reducing fuel loads of cheatgrass (*Bromus tectorum*) and other annual grasses. Early spring grazing can reduce annual grass biomass and seed production (Mosley, 1996; Mosley and Roselle, 2006). Prescribed fire can then be applied with less damage to desirable plants and less risk of fire escape and collateral damage. The prescribed fire destroys any remaining seeds in the litter layer and livestock grazing the following spring will control new seedlings of cheatgrass (Diamond et al., 2012). Integrating fire with targeted grazing provides more complete destruction of seeds without the necessity of repeated grazing, which can be difficult to accomplish on large areas.

Grazing and Mechanical Control

When the goal is controlling woody plant species or shrubs, sheep may require mechanical assistance to get the job done. Trees and shrubs may be too tall for sheep to access and mechanical treatment can be used to lower the canopy or provide an initial top-kill of the plant. Regrowth of shrubs and trees is generally more palatable and contains fewer toxins, so it is readily consumed by grazing animals (Campbell and Taylor, 2007). Targeted grazing can also be combined with mowing for restoration. Sheep will avoid consuming decadent, mature plants, particularly grasses. Mowing to knock down tall plants combined with sheep to graze regrowth may provide an effective means of suppressing undesirable tall forbs and grasses (Krahulec et al., 2001).

Grazing and Insect Biological Control Agents

Numerous purposeful introductions of insect biological control agents have been employed in an attempt to control the ever-expanding populations of invasive plants in the United States. However, only a handful of these agents have had the desired effect on the target plant (McFayden, 1998) and decades may be required for the insect populations to reach a density that negatively impacts the target plant (Story et al., 2008). Often, multiple agents are introduced that feed on different plant parts such as leaves, stems, or roots in an effort to intensify damage to the target plant (Müller-Schärer and Schroeder, 1993). Grazing by sheep can contribute to that damage and improve the efficacy of introduced biological control insects by: 1) reducing the seed dispersal of the target plant (Frost et al., 2011, 2012; Wallace et al., 2008); and 2) altering the age structure of the plant community to make it more susceptible to insect biological control agents (Olson et al., 1997). Olson et al. (1997) demonstrated that 3 yr of successive sheep grazing in a spotted knapweed infested plant community resulted in an increase in the average age and size of knapweed plants. These older plants support large taproots that are more susceptible to attack by the root feeding weevil *Cyphocleonus achates* (Story et al., 1996).

Conversely, sheep may reduce biological control populations through direct consumption of eggs and juvenile insects on leaves and in seedheads as well as by trampling eggs or adults hiding or overwintering in leaf litter (Winston et al., 2010).

Currently, most biological control handbooks urge against grazing in areas where insects have been released or advise grazing when insects are not vulnerable to consumption or trampling by grazing animals (Winston et al., 2010). These concerns prompted a study to examine effects of targeted sheep grazing on biological control insects of spotted knapweed. Abundance of *Cyphocleonus achates* (a root boring weevil), *Larinus minutus* and *L. obtusus* (seedhead feeding weevils) was measured in response to targeted sheep grazing applied at a moderate grazing intensity (0.8 ha·AUM^{-1}) and high stock density (38.5 yearling sheep·ha^{-1}) in either July or August (spotted knapweed in late bud-early flowering stage vs. full flowering stage). Three years of targeted sheep grazing in either July or August did not affect *Cyphocleonus* or *Larinus* abundance, but negative effects on spotted knapweed reproduction were additive (Table 1). Combining biological control insects with targeted sheep grazing reduced viable seed production by 98% compared with biological control insects alone, and *C. stoebe* seedling density was reduced 92% by grazing in July, and 63% by grazing in August, compared with biological control insects alone (Frost et al., 2012). Finally, the levels of seed production with biological control insects combined with targeted sheep grazing were far less than the estimated amount needed to sustain spotted knapweed populations (Story et al., 2008), whereas the amount of seed production with biological control insects alone was far more than the minimum amount required. Story et al. (2008) reported that insect biological controls were responsible for large knapweed die-offs in Montana; however, it took 30 yr for the insects to attain sufficient populations to exert an impact alone. Thus, addition of sheep grazing to the biological control insects is depleting the seed bank at a more rapid rate, thereby lowering the ability of the knapweed population to respond positively to future disturbance or favorable environmental conditions, theoretically reducing the time required to realize population declines of spotted knapweed.

Similarly, Jacobs et al. (2006) reported that multispecies grazing (sheep and cattle) at a moderate stocking rate on leafy spurge-infested rangeland did not suppress populations of *Aphthona* beetles (leafy spurge biological control insect). These researchers further concluded that combining cattle and sheep grazing with *Aphthona* beetles reduced leafy spurge density and cover more than *Aphthona* alone. Additionally, leafy spurge seed productive ability was reduced by about 94% after 5 yr of grazing in either a continuous or rotation system.

These research projects demonstrate the success of integrating above-ground grazing damage by livestock with above- and belowground attacks by insects on suppression of invasive weeds. Consequently, grazing should be considered a viable management addition to rangeland areas where insect biological control agents have been released for either leafy spurge or spotted knapweed suppression, particularly on public lands where integrated management is promoted.

Implications

The future of sheep production may well depend on the ability of the producer to sell the ecological value of sheep as vegetation management tools as well as the meat and fiber products produced. As part of an integrated restoration plan, sheep grazing can be successfully used to increase the efficacy and longevity of more expensive vegetation management tools, while providing an economic return from rangelands. Additionally, using sheep grazing as one of the tools in an integrated management program, rather than the sole treatment, goes farther in convincing land managers that the livestock are there to achieve vegetation management rather than an attempt to increase stocking rate or obtain less expensive forage. Furthermore, the integration of several management techniques can provide more complete control than a single method used alone, as well as more rapid progress toward vegetation objectives resulting in more economical control. Sheep grazing has been successfully integrated with herbicides, mechanical treatments, prescribed burning, and insect biological control agents to improve the success of weed management, and to sustainably produce, meat, fiber, and other products from sheep grazing on rangelands.

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Table 1. Mean (n = 3 yr) response of insect biological control agents and spotted knapweed reproduction after integrating targeted sheep grazing and biological control insects on foothill rangeland in western Montana.

	Density (number/m^2)			
Herbivory treatment	*Cyphocleonus achates*	*Larinus* spp.	Viable spotted knapweed seeds produced	Spotted knapweed seedlings
Biocontrol + sheep in July[1]	12 [a]	3 [a]	4 [a]	6 [a]
Biocontrol + sheep in August[2]	8 [a]	4 [a]	8 [a]	26 [b]
Biocontrol insects only	6 [a]	4 [a]	275 [b]	71 [c]

[1] Sheep grazing applied when spotted knapweed in late bud-early flowering stage.
[2] Sheep grazing applied when spotted knapweed in full flowering stage.
[a,b,c] Column values with different superscripts differ ($P \leq 0.05$)

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2013 Sheep Symposium[1]
ADVENTURES IN THIN MARKETS, CONTRACTING AND CONCENTRATION: TODAY'S LAMB MARKET

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ABSTRACT: The lamb market in 2012 was a disaster as lamb prices collapsed more than 50% from record highs early in the year. The collapse spurred searches for reasons why prices collapsed and to identify those responsible for the collapse. However, market analysts and producers with a longer historical view of the industry couldn't help but have the feeling that we have been here before. This paper examines the lamb market leading up to the price collapse, causes for lower prices, and where the industry might go from here.
Key Words: lamb price, marketing, price collapse, sheep industry

"Its déjà vu all over again"
– Yogi Berra

Looking Back

The U.S. sheep industry has had a long history of declining sheep numbers. The reasons for the decline are well known, including increasing production costs, declining demand for lamb, imported lamb, competition from man-made fibers, declining wool demand, and others.

Recently, there have been reasons for optimism in the industry as lamb prices increased, along with other meat prices. Wool prices were recently at the highest since the late 1980s. While the industry was smaller, growing ethnic markets for lamb, new interest by high-end niche markets, and branded products were providing alterative marketing opportunities. The industry had also begun concerted efforts to get producers to expand their flocks in hope to maintain a critical mass of lambs for the meat packing infrastructure.

Anatomy of the Crash

Prices for live lambs and lamb meat had increased sharply to record high levels during 2011 and early 2012 (Figure 1). Record-high prices certainly caused the demanded quantity of the product to decline, especially during a time of slow economic growth following the Great Recession and relatively lower competing meat prices. As consumers pushed back against high lamb prices, retailers saw sales slip as the product did not move off the shelves. That pushed retailers to buy less lamb, pressuring packers and wholesalers to realize that they could not move product at the historically high prices. As they knew that they had to buy lambs lower, they worked to "jawbone" producers that lamb prices had to fall, to expect lower lamb prices. Yet, given that packers make more money by buying the product cheaper, producers expect them to always argue that lamb prices are too high. Another result of the high prices and expectations of high prices was that cold storage stocks of lamb grew, especially high end cuts like racks, in part because they could not sell them, but also in part due to speculation that the racks could be sold into the strong and rising market that no longer existed. Lambs for custom slaughter and processing that had small defined markets continued to move through the system at normal weights, while lambs in feedlots did not move, creating a backup in the marketing system.

Lamb feeders serve an important role in the industry by providing the mechanism to spread out lamb supplies throughout the year. Whether due to feeders not wanting to sell lambs to packers at the offered lower prices, or packers not wanting the lambs, the result was that the lambs on feed in feedlots continued to eat, gaining weight and age as time passed. Figure 2 contains lamb and yearling dressed weights that indicate the heavy weights in mid-year 2012. Packers that owned lambs on-feed also kept feeding their animals well beyond normal end-points. This caused 2 problems: 1) increased tonnage in the market; and 2) over finished, very fat, heavy lambs that resulted in older, more aged lamb that was of poor quality, which negatviely affected taste. Some of this over fat, poor quality lamb entered the market further damaging lamb demand as customers were unhappy with the product.

As lamb feeders continued to lose money, new lambs came to market and the lower finished lamb prices were passed on to producers in the form of sharply lower feeder lamb prices. Figure 3 contains feeder lamb prices for a 3-market average of Colorado, Texas, and South Dakota prices. Feeder lamb prices declined from over $210 per cwt in the spring of 2012 to $90 per cwt by July. As lamb prices declined, the extent of the corn belt drought became apparent and rising feed costs further ate into feeder lamb prices.

Overall, a market correction that began in the retail segment of the industry with struggling demand, led to an

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even stronger downward spiral in prices as the different segments responded in their own interests.

It's important to remember that the industry has been here before. The market situation that led to a large number of over finished, heavy lambs that weighed the market down through volume of meat and/or poor quality has happened before in the U.S. lamb industry. The same situation occurred in the late 1950's, spurring the Congressional study of marketing in the industry (NWGA, 1960). Additionally, the market collapse in the late 1980's prompted the industry to fund a study of the lamb market and recommendations for how to improve the industry marketing system. The study was the oft-cited "Texas A&M Study" (TAMRC, 1991). Again in 2001, falling lamb prices led to over feeding that forced prices even lower (McKinnon, 2001).

That the same situation has happened time and again in the industry, should suggest that there are repeating factors at work in the market and, indeed, there are. The workings of the lamb market reflect some classic economic problems. It is important to note that the lamb market is not unique in experiencing these problems.

First, lamb is a non-storable commodity. Lambs continue to eat, get fatter, over-finished, and age. They can't be stored "on the hoof" nor can the meat be stored for long periods of time in cold storage. Non-storability sets the stage for a product that must be sold at whatever the market will bear, even if that is into a collapsing market.

Second, the lamb market can also be characterized as a concentrated market. Few packers mean that there are few buyers of lamb from producers and that the potential for exercising market power to lower prices to producers may exist. We might also think of the lamb industry as concentrated in another way – the industry is a lot smaller than it used to be.

Third, the various industry segments – producers, feeders, packers, wholesalers, and retailers – are not closely tied together. They are not integrated or tightly coordinated as would be found in the poultry or hog industries. It is not a "cost-plus mark up" business from one segment to the other. Therefore, it is not often that all segments of the industry profit at the same time. A sector (i.e. packing) profits from buying lambs cheap and/or selling meat high. Buying lambs cheap forces losses onto the producer or feeder. Selling the meat high forces losses onto the buyer. So, one sector can and will lose money, while another sector is profitable. Additionally, in the case of 2012, a few other factors can be considered, such as the overall economy, relative prices of other meats, feed costs, and drought. Each of these contributed to the price collapse of 2012. The price decline in 2012 was not limited to the United States. The 2012 New Zealand lamb market was reported to have similar problems (Morgan, 2012).

In past years, imported lamb has had a large negative effect on U.S. lamb prices. However, 2012 was likely not one of those years. Lamb imports declined as a percent of U.S. lamb production for the year. It is true that imported lamb is less expensive than domestic lamb, and that it most likely provided some competition for domestic lamb that was at a record high price. But, the volume imported did not show growth over the year before.

Where to Go from Here

The USDA sheep inventory report, released in February, 2013, indicated that the number of sheep and lambs declined about 1 percent over the previous year (USDA, 2013). Considering the magnitude of the lamb price collapse, the severity of the drought in Texas and the Great Plains, and higher costs, the inventory report was a good one. Sheep number in Texas actually increased. That confirmed anecdotal stories that sheep inventories may have come through the drought in better shape than cattle inventories. It also confirmed other anecdotal information relating to increasing interest in lamb production in Central Texas as that region looks into tapping into the growing niche markets for local lamb in urban areas.

The industry continues to face serious challenges including public land use, drought, a lack of predator controls, and high feed costs. History indicates that past problems with over finished, poor quality lambs contributed to a struggle to rebuild lamb demand after consumers have purchased poor quality products. Consumers also face choices at the retail meat counter and restaurants between lamb that is priced higher than beef, which is also higher priced relative to chicken. There is evidence that when consumers don't buy a product due to high prices it is difficult to get them back.

The 2012 market crisis highlighted a need for more market information. This market information may include changes in grades, standards, and market definitions for lamb. For example, at what point is a product sold as lamb no longer lamb but mutton? The relationship between age and taste profile is an important issue for lamb demand. Improved definitions of products in the market place would benefit producers and consumers.

Another area for market improvement is in lamb price information, especially by expanding the number of weight breakdowns in the price reports. Prices for heavy, over finished lambs could be reported in a separate category allowing for market information on price discounts for those lambs relative to lighter, younger lambs. Some markets for lamb are growing. Ethnic markets, largely made up of Muslims and immigrants from parts of the world where more lamb is consumed, are growing. Recent survey work highlighted differences in these market areas and indicated ways to expand or target lamb sales to these audiences (TAMRC, 1991). Lamb also appears to have an opportunity in the local, "naturally grown" type of market segment. Even in the ethnic lamb consumer study, consumers viewed lamb positively as a "natural product".

Maintaining a critical industry mass will continue to be a concern. Fewer sheep and lambs mean it is more difficult to maintain a critical mass of lambs available to keep a packing plant open. Recent consolidation and packing plant closing in the industry has resulted in further concentration.

The issue of industry coordination remains an issue that will continue to affect price. It will remain an issue as long as each sector of the industry is separate and profits when other sectors are unprofitable. This is not unusual in agriculture. Improved coordination could prevent or limit

over finished lamb problems that have plagued the industry throughout recent history. The TAMRC (2001) study went into great detail about the need for the various industry segments to better coordinate. The report also outlined an action plan for the industry. One of the most important industry accomplishments following that report was the establishment of the lamb checkoff. However, one can't ignore that in some cases more industry coordination resulted in other problems as seen in hog and poultry industries. Given the structure of the lamb market, it is difficult to envision at this point in time a more cooperative environment.

Summary

The sheep industry has been in the current market crisis before. However, this time the industry is much smaller, and recovering from low prices may require different alternatives than have been utilized in the past. Maintaining a critical mass is an even bigger concern as the sheep industry looks to the future. The classic economic problems that have been observed in many industries remain. However, on the positive side, lamb prices have been recovering since the market crash of 2012. Lamb weights are down, reducing lamb supplies and eliminating the over finished lambs from the market. Competition from other meats remains, as does the effect of the recession on consumer incomes. Rebuilding consumer demand should be expected to take time. Fortunately, ethnic markets are rapidly growing, which appear to be providing new nontraditional markets for U.S. lamb.

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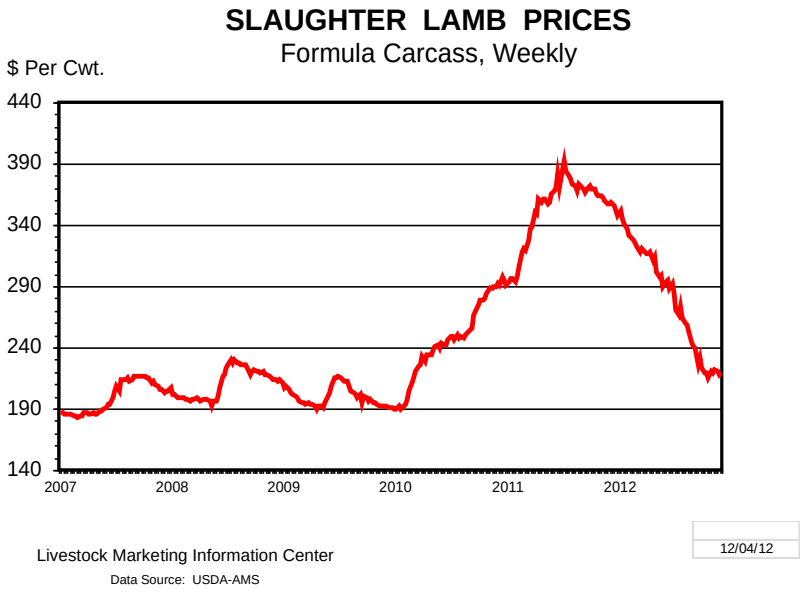

Figure 1. U.S. Slaughter Lamb Prices, 2007-2012

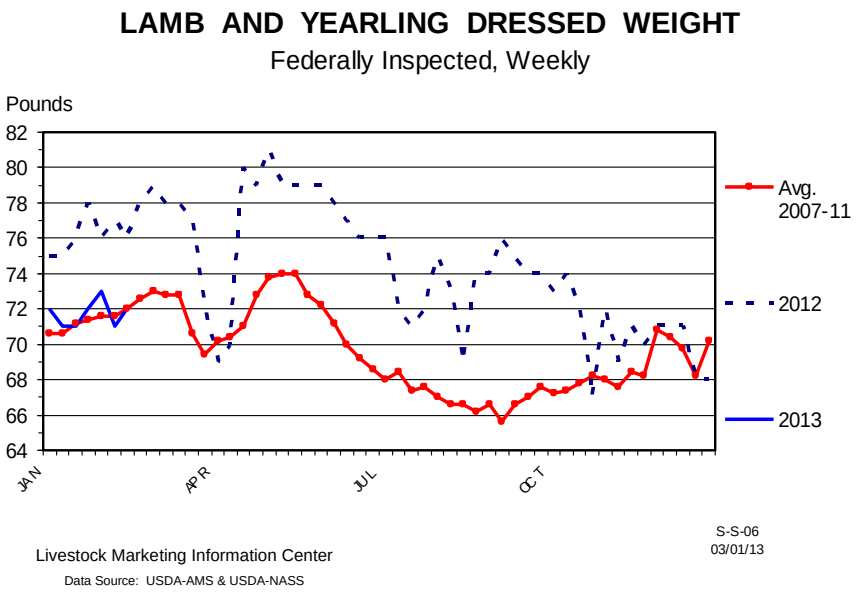

Figure 2. U.S. Lamb and Yearling Dressed Weights

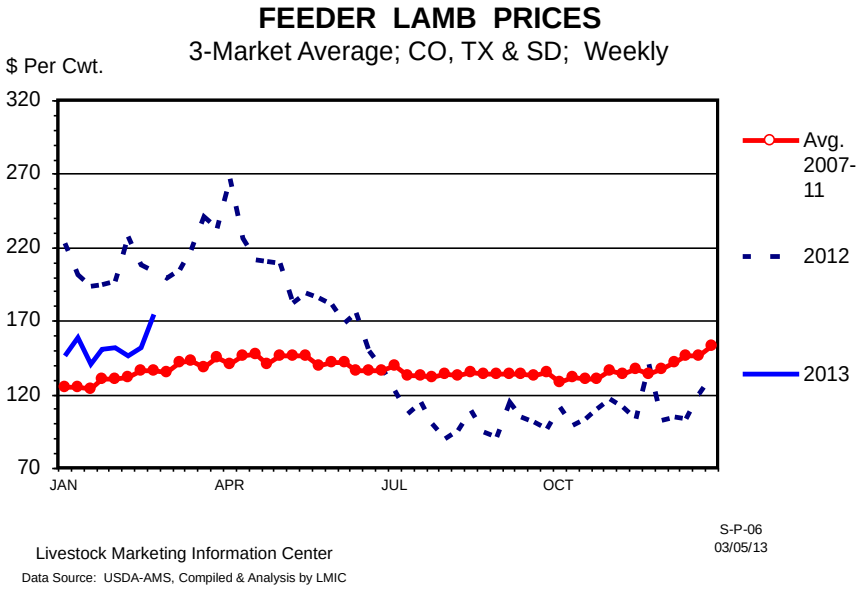

Figure 3. Average U.S. Feeder Lamb Prices

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2013 Sheep Symposium[1]
SHEEP, BLACK SWANS, AND THE FUTURE OF AGRICULTURE

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ABSTRACT: The U.S. sheep inventory continues to decline, regardless of recent stimulus programs and campaigning strategies. Only creative, out-of-the-box efforts that effectively educate, facilitate, and recruit prospect sheep producers can reverse this devastating decline. The purpose of this presentation is to examine the longstanding dogma of what has contributed to the decline of the industry, and present a unique case of how labor force enlightenment could possibly have a positive effect on sheep production in the U.S. Compared with other food animal production species, sheep generally require more labor; the dogma is that available labor is lacking. However, there is a segment of society that potentially has unused labor and that appears to be in need of unique opportunities that may align with their ideals; this segment should be targeted for expanding sheep numbers in the U.S. The potential labor force is retiring baby boomers, which is a rapidly expanding demographic. Industry promotion efforts should be focused on educating baby boomers on the economic, ecological and lifestyle values of sheep production.
Keywords: agriculture, baby boomers, food, sheep industry, sustainability

Introduction

The sheep industry is in a crisis and has been for a long time. As I reviewed previous reports on problems and solutions for the sheep industry, it was obvious that no new long-term solutions have been proposed in the last 30 yr; what has been attempted in the last 50 yr, has not worked (Gee et al., 1977; CAST, 1982; Parker and Pope, 1983; Purcell, 1998; Jones, 2004; Williams, 2008). In recent times, more often than not, it is agricultural economists that are describing problems and suggesting the solutions. However, it appears that the sheep industry violates the basic premises of economics; i.e., neither producers nor consumers appear to be rational agents. My premise in this paper is to develop a convincing rationale for a new solution to recommend to the industry, while it still has some infrastructure left. Please be aware that this paper is written with a great deal of trepidation, because I do not want to offend people that I respect or an industry that afforded me a wonderful career. Furthermore, while the following maybe wrong, I hope that my arguments, at the least, stimulate discussion that results in a reversal of a long-term decline and ultimate collapse of the U.S. sheep industry. With this said, I go forward with confidence in knowing that while I may not do any better than those that preceded me, I cannot do much worse.

State of the Sheep Industry

Having faced and endured many problems sheep producers appear to be in a state of learned helplessness. This is a condition defined as the general expectation that one cannot control important events, leading to lowered persistence, motivation, and initiative. Seligman and Maier (1967), while studying the relationship between fear and learning, discovered an unexpected phenomenon, while doing experiments on dogs using Pavlovian (i.e., classical) conditioning. In Seligman's experiment, he paired a tone with an electric shock, restraining the dog in a hammock during the learning phase. The idea was that after the dog learned to associate the tone with the shock, the dog would feel fear on the presentation of a tone, and would then try to escape. The conditioned dog was placed into a shuttlebox, which consists of a low fence dividing the box into 2 compartments. The tone was sounded, but surprisingly, nothing happened. The dog was expected to jump over the fence. Next the conditioned dog was shocked, and again nothing happened. The dog just laid there. When an unconditioned (i.e., normal) dog was put in the shuttlebox the dog, as expected, immediately jumped over the fence to the other side upon receiving a shock. Apparently, what the conditioned dog learned in the hammock was that trying to escape from the shock is futile; this dog learned to be helpless. The theory of learned helplessness was then extended to human behavior, providing a model for explaining depression. Depressed people learned that all action is futile.

Declining sheep numbers are a sign of learned helplessness in the sheep industry. Consider several studies have shown that sheep numbers are not responsive to positive returns on investments. For instance, between 1972 and 1987 sheep had positive receipts less cash expenses for 15 of the 16 yr (Figure 1) and these positive receipts increased 460% over that period (Stillman et al., 1990). However, during that same period, stock sheep inventories fell 1.5 percentage units per year for a loss of 24 million head. Similarly, a study of 5-yr breeding ewe elasticity to revenue exhibited a 1% increase in lamb prices would result

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in only a 0.7% increase in breeding ewe numbers (Jones and Schroeder, 1998).

Elasticity is used to assess the change in one variable (e.g. ewe numbers) as a result of a change in another variable (e.g. lamb prices). When the value is greater than 1, this suggests that the demand for the good/service is affected by the price, whereas a value that is less than 1 suggests that the demand is insensitive to price. The elasticity found by Jones and Schroeder (1998) was significantly lower than a similar study using economic data from 1924 to 1983, which had a 5-yr elasticity of 1.4 and included a period of increasing sheep inventories and peak sheep numbers in the mid 1940's (Whipple and Menkhaus, 1989). Williams et al. (2008) reported returns to labor, investment, and risk on sheep operations of 17% in 2006, but the U.S. breeding sheep inventory for January 1, 2013 reported a 13% decline since 2006.

Jones and Schroeder (1998) also found a very low elasticity associated with wool revenue and predicted that elimination of the wool subsidy program would likely have only a small impact on the size of the industry. However, that was not the case. The loss of the wool price supports between 1992 and 1994 led to a major sell-off of breeding stock (Williams et al., 2008). It appears that from an economic perspective the sheep industry overreacts to negative consequences and under reacts to positive consequences, which could be interpret as a sign of learned helplessness in the industry.

Like sheep producers, lamb consumers are relatively insensitive to prices as indicated by several studies that have estimated low elasticities between lamb price and per capita lamb consumption (Williams et al., 2008). In the U.S., lamb has always been a specialty meat consumed primarily by ethnic groups and discerning consumers rather than a staple food item (Figure 2). Over the past 100 yr, annual per capita lamb consumption on a carcass basis has not exceeded 3 kg (6.6 lbs.) per person and has always vied with veal for the last place meat item of the American diet. Per capita consumption of lamb was about 0.6 kg (1.3 lbs) between 1975 and 1995 whether the price was $2.60 per kg ($1.18/lb) or twice that at $5.20 per kg ($2.35/lb), respectively.

Problems in the Sheep Industry

Producers, economists, and animal scientists each have theories to reverse the decline in sheep numbers, which are:

- Sheep producers believe predator control is the solution.
- Economists believe that increasing demand and thus price is the solution.
- Animal scientists believe that increasing production efficiency, i.e, lowering production cost, is the solution.

Predator Control

Predation is unique to the U.S. as compared with causes for sheep number declines in other countries (Woodford, 2010). A recent survey of sheep producers reported that "better predator control" was the fourth ranked reason that would help producers to expand their sheep numbers. However, the ranking varied by region and flock size and was less important in small flocks and regions were sheep numbers are currently expanding (ASI, 2010). However, I would argue that predation is not the problem, labor is. Some producers are able to successfully produce lamb in the face of high predator densities. To accomplish this, they committed to using available technologies (e.g., trapping and snaring, livestock protection dogs, night penning, etc.) to ensure the safety of their livestock. Producers have shown that this is possible to suppress predators below an economic threshold. But, the commitment required more and persistent labor than most producers were willing to invest. Finally, I would leave the reader with a quote from Shimon Peres, current president of Israel, to ponder relative to the predation problem: "If a problem has no solution, it may not be a problem, but a fact – not to be solved, but to be coped with over time." .

Increasing Demand and Price

"If demand was increased and thus, the price of lamb and wool, it would solve the industries problems" is a common statement by members of the sheep industry. Clearly, at some price level, sheep inventories would increase for a sufficient duration to make a real difference in the industry. But, as demonstrated by the low elasticities between revenue and ewe inventories, the price at which that would occur would be greater than could realistically be expected, which would not be sustainable. Domestic production cannot meet the current demand for lamb. In spite of econometric studies to the contrary (Williams et al. 2010), there is little evidence that promotion has helped reverse the downward trend in sheep numbers. Thus, increased demand would marginally benefit U.S. producers, but rather, would be of greater benefit to Australian and New Zealand producers. Finally, when slaughter lamb prices are high, "over feeding" of lambs in the feedlot is incentivized, resulting in a rapid devaluation of fat lambs. This happened in 1988, 2001 (McKinnon, 2002) and 2012, and it resulted in a negative perception by the producers, which, as stated above, caused an overreaction.

Increasing Production Efficiency and Reducing Labor

Efficiency of sheep production has increased, albeit more slowly than other livestock species (Parker and Pope, 1983). Sheep producers have been rather slow adopters of new technologies, and if sheep numbers continue to decline, availability of new technologies to further improve efficiency of sheep production will be reduced. Purcell (1998) concluded that it will be very difficult to solve the viability problems of the sheep industry based solely on increases in production efficiency. This is particularly true now that half of the lamb consumed in the U.S. is imported and domestic producers would most certainly loose a price war.

Sheep have many biological advantages compared with beef cattle relative to converting forages to high quality protein because of the greater relative growth rate of lambs

and greater prolificacy of ewes (Walker, 1994). Sheep can better utilize steep rangelands with low forage production than cattle. But, sheep have a major cultural disadvantage. Sheep production requires more labor per animal unit than cattle enterprises (Stillman et al., 1990; Woodford, 2010). The additional labor required for sheep production is often cited as an obstacle to expansion of the industry in terms of hired labor and shearers. The reality is that this additional labor requirement has an equal or greater toll on sheep enterprise operators and their families. For example, as older operators seek to reduce their work load or the business passes to the next generation, whose cultural expectations include greater leisure time, sheep are often the first enterprise to be abandoned. This problem is exacerbated because of the highly concentrated nature of the sheep industry, where 1.5% of the operations account for 48% of the national flock (Williams et al. 2008). It is these large operations where labor from both the operator and hired labor is most crucial for a successful business. This is why I argue that labor is the resource most limiting to resurgence in the sheep industry. However, I contend that the amount of work required to successfully operate a large range sheep operation is no longer normative in this country. Further, only the families with a strong sheep culture and great equity will be able to maintain these operations in succeeding generations.

Agriculture, as it is practiced in developed countries, relies on large inputs of fossil fuels. A comparison of corn inputs and yields between mechanized and labor-intensive production systems demonstrates this point (Pimentel, 2009). Mechanized corn production requires 11 h of labor and 8,228 Mcal of fossil energy inputs to yield 9,400 kg/ha (150 bu/acre) of corn. Labor intensive corn production requires 634 h of labor and 4,082 Mcal of fossil energy inputs to yield 1,721 kg/ha (27 bu/acre) of corn. The fossil energy in the mechanized system is more efficiently used in producing 4 Mcal in crop output for each 1 Mcal of fossil fuel input compared with a ratio of 1:1.5, respectively, for the labor intensive system. This comparison illustrates 2 important points relative to modern agriculture and societal norms. Agriculture has progressed through intensification and the substitution of fossil energy for human labor, and both trends appear to be universal for all types of production. Because sheep production, and particularly range sheep production, has fewer opportunities to intensify or substitute fossil energy for labor, it will be at a competitive disadvantage to food production systems that can intensify, at least as long as fossil energy is relatively abundant. However, should fossil fuels and/or feed grains become limiting, sheep production could become very competitive as a source of high-quality animal protein. In that situation, lamb would have a clear advantage over beef because acceptable lamb can be produced on forages and requires only about 25% as much fossil energy to produce as feedlot finished beef (Cook, 1976).

The current situation of high grain and fuel prices is not new. In the mid 1970's, following the 1973 oil embargo and concomitant high grain prices, there was much interest and research on forage finishing beef. The concern was that food production would not match population growth and that feeding grain to livestock would have to end. Of course those concerns passed, at least until recently, and world population has increased from 4 billion in the mid 1970's to its current 7 billion. However, the norm, until the Green Revolution in the 1950's, was that feed grains were too scarce to support livestock feeding on today's scale. It is impossible to say if the current situation will last, because as Nobel physicists Niels Bohr famously said: "Prediction is very difficult, especially if it's about the future."

Future of the Sheep Industry

One trend that does not require prediction, because the demographics are already in place and are projected to have a large impact, is the collapsing birthrate in the developed world (Drucker, 1999). In all developed countries, the strategy of all agricultural institutions will have to be based on a different assumption of a shrinking population, and especially of a shrinking young population. This development could turn the U.S. industry around, particularly if a concomitant decline in availability of feed grains occurs. One of the implications of an aging population is that people will have to continue to work to older ages. Most of them will not have worked in physically demanding occupations and will be able and perhaps prefer occupations that require a moderate level of physical activity. Also, because wealth tends to be concentrated in the older generation it is likely that many of the baby boomers will have equity in small rural properties. Is anyone starting to see a match here?

The Black Swan

Nassim Taleb (2010) defines black swan events as an event that is a surprise (to the observer), has a major effect, and after the fact is often inappropriately rationalized with the benefit of hindsight. Examples of black swan events include the rise of the Internet, the personal computer, and the September-11 attacks. The black swan that could have a major impact on sheep numbers would be an agricultural policy that emphasizes agricultural and environmental sustainability over production and the regulation of externalities (i.e., a consequence of an economic activity that is experienced by unrelated third parties). For example, consider air pollution and regulation of waste discharge from manufactures. Unless regulated manufacturers can discharge waste into the atmosphere with no cost to either the manufacturer or the direct consumer of the goods produced, all users of the air must ultimately pay the cost (e.g., such as increased health care cost). Externalities can be regulated by either fiat or by trade (e.g., carbon credits). Sustainability and negative externalities are 2 sides of the same coin. Sustainable agriculture systems do not produce negative externalities and, in fact, often produce positive externalities (i.e., ecosystem services).

The first notable effort by the American Sheep Industry Association to promote environmental benefits of sheep grazing occurred in 1994 with the publication of a special issue of the Sheep Research Journal: "The Role of Sheep Grazing in Natural Resource Management". This was followed by publication of: "Targeted Grazing - a natural approach to vegetation management and landscape

enhancement" (Launchbaugh and Walker, 2006). Unfortunately, this effort has not been widely embraced as a method to increase sheep inventories, perhaps because it is more labor intensive than raising sheep strictly for commodity purposes. Currently, only about 6 percent of sheep producers are paid to provide the environmental benefit of weed control (ASI, 2010). If the ecological benefit of sheep grazing was appreciated and the economic benefit compensated, it could be the black swan that enables the phoenix to rise again.

Implications

The biggest problem of the sheep industry is declining inventory. This is not just a U.S. problem, but it is happening in all developed countries (Williams, 2008; Woodford, 2010). If this continues to happen, infrastructure required to support the industry will shrink and the problem of concentration of packers and buyers will intensify. Large operations must be sustained; this is where the sheep are currently concentrated. However, growth will be in smaller operations. A trend has already begun. The growth will be with unique breeds rather than traditional dual purpose breeds. The industry should make a concerted effort to recruit retiring small-acreage-owning baby boomers to the sheep industry. Sheep production could be a reasonable fit for that segment of the population. Furthermore, sheep production matches the concept of niche markets as a growth area for the industry; this is especially true in using sheep for ecosystem restoration and improvement. Additionally, sheep production provides locally grown food, which coincides with the ideals of many baby boomers. I suggest that promotion dollars could be better spent on advertising the virtues of sheep production in forums such as the AARP magazine rather than the culinary delights of lamb in food magazines. Make no mistake, the only metric that matters relative to the sheep industry is the effect of new programs and technologies on the size of the U.S. sheep flock. As Rodney and Sharon Kott head off to retirement, maybe they should be the "poster people" for this new segment for expansion in the sheep industry.

This solution may seem rather weak in comparison to the problems faced by the industry, but I believe that it is at least in the right direction. "The mere formulation of a problem is far more essential than its solution, which may be merely a matter of mathematical or experimental skills. To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks real advances in science."--Albert Einstein. I hope that I have accomplished this.

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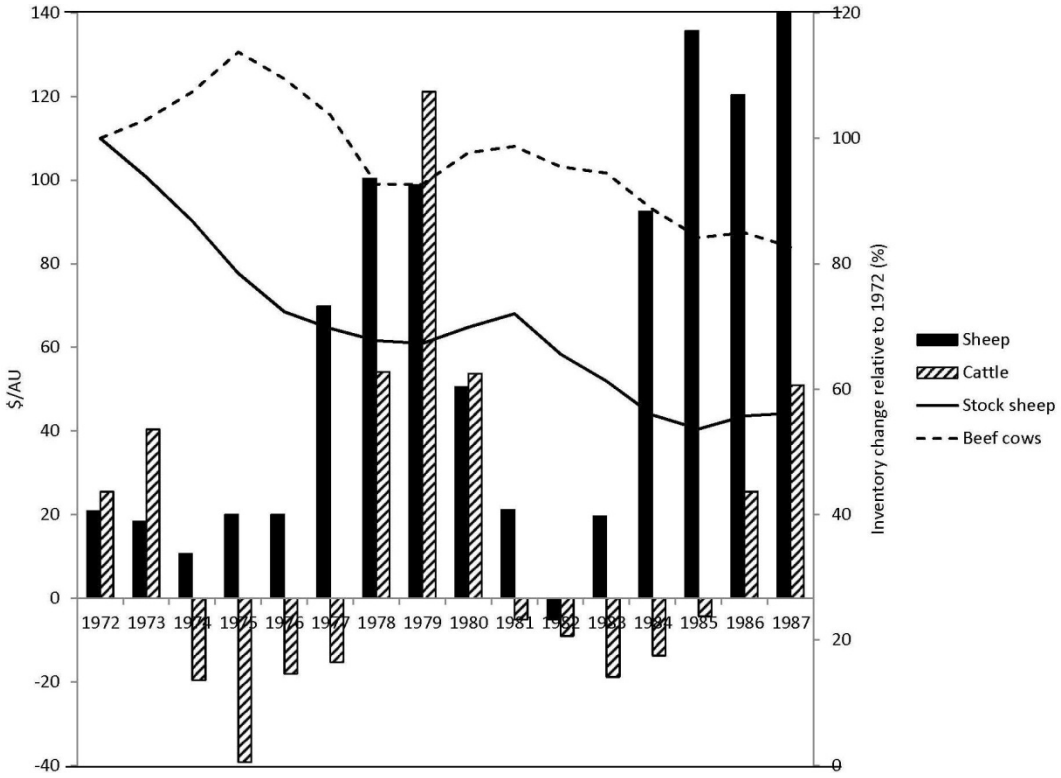

Figure 1. Comparative returns (bars) and inventory changes (lines) for sheep and beef cow enterprises. Returns are cash receipts less cash expenses (Stillman et al. 1990). Inventory changes for stock sheep and beef cows are shown relative to 1972 inventory where 1972 inventory = 100.

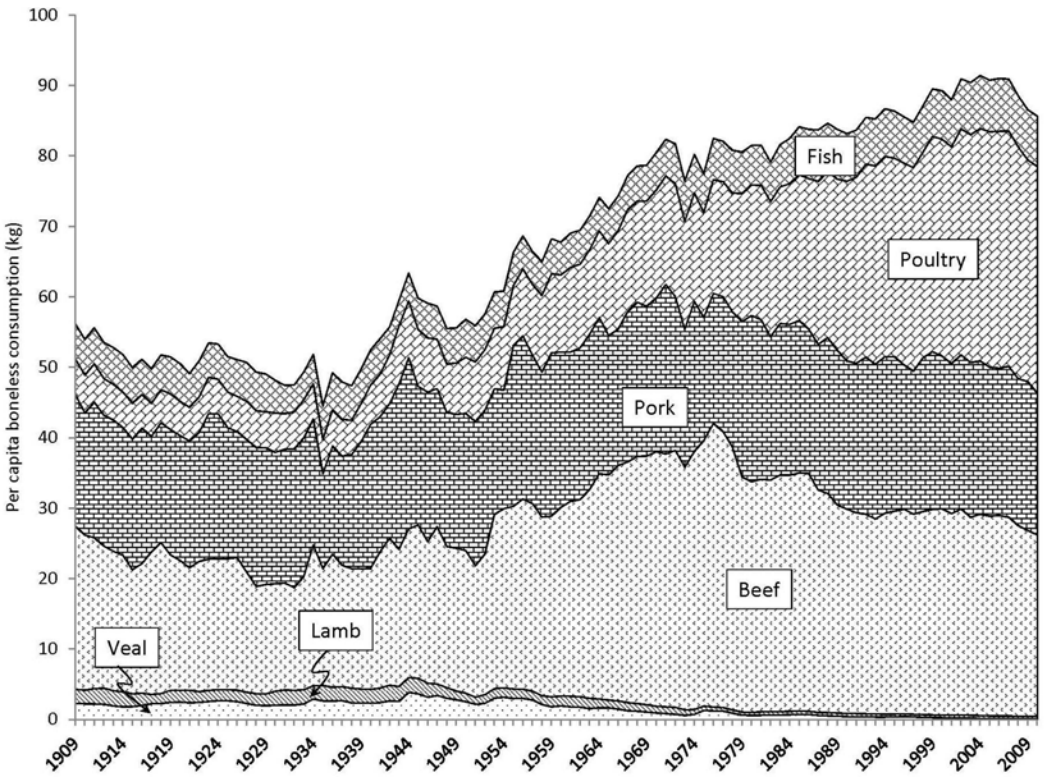

Figure 2. Boneless per capita consumption of different animal protein sources.

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EFFECTS OF ON-ARRIVAL, DELAYED VACCINATION AND SUPPLEMENTAL LYSINE ON PERFORMANCE, ANTIBODY TITER, TEMPERATURE AND METABOLIC PROFILES IN RESPONSE TO MODIFIED-LIVE VIRAL RESPIRATORY VACCINATION

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ABSTRACT: The objective of this work was to evaluate effects of timing as well as supplemental lysine associated with the administration of a modified-live respiratory viral vaccine (IBR, BVDV, PI3, BRSV) on performance, feed intake, antibody titer response, and febrile response. In experiment 1 thirty-36 (Angus and Angus crosses; initial BW = 265 ± 20 kg) were randomly assigned to treatments (3 pens/treatment with 4 heifers/pen), which included no vaccine (CON), vaccination on d 0 (DO), and a delayed vaccination on d 14 (D14) of the receiving period. Heifers were fed in 6 x 12 m pens equipped with GrowSafe feeding systems. Daily feed intakes were recorded and BW measured weekly. Temperature data loggers were attached to a blank controlled intrauterine drug release devices (CIDR; contained no active compound) that recorded vaginal temperatures every 5 min for the experiment; vaginal temperatures were then averaged for every h before data analysis. All data were analyzed using pen as the experimental unit. No differences ($P > 0.10$) among treatments were observed for initial BW, final BW, ADG for d 0 to end, or overall G:F. A treatment x d interaction ($P < 0.05$) was observed for feed intake. Daily intake was decreased for D14 versus D0 on d 14 ($P < 0.01$) and 15 ($P < 0.10$) and decreased ($P < 0.05$) on d 15 for the average of vaccinated calves versus CON. Eating rate (grams consumed/eating duration) was decreased ($P < 0.05$) on d 14 for D14 versus D0. No differences ($P > 0.10$) among treatments were noted in the number of eating events/d. A treatment x d interaction ($P < 0.01$) was observed for vaginal temperature. Vaginal temperature was increased ($P < 0.10$) on d 1 for D0 versus D14 heifers and increased for D14 versus D0 on d 14 ($P < 0.01$), 15 ($P < 0.05$) and 16 ($P < 0.05$). In the second experiment, 64 neonatal Holstein bull calves (7 ± 2 d of age; BW = 37 ± 4.2 kg) were used in a completely randomized design to evaluate the effects of supplemental lysine. Calves were fed milk replacer supplemented with either 17 g/d L-lysine monohydrochloride (LYS; 28 calves) or an equivalent amount of casein (CAS; 28 calves) for 42 d. Calves were then vaccinated with either an IN IBR-parainfluenza virus-3 (PI3; Nasalgen, Merck, Summit, NJ) or an IM (IBR-PI3-bovine viral diarrhea type I and II, bovine respiratory syncytial virus; Express 5, AgriLabs, St. Joseph, MO) modified-live vaccine on d 36. A control group (8 calves) received no supplement or vaccination. All calves were housed in individual calf pens (1.2 x 2.1 m). Daily feed intakes were monitored and BW measured weekly. Calves were bled on d 0, 35, 36, 37 and 42. Temperature data loggers were attached to rectal probes and temperatures were recorded every 5 min from d 28 to d 42. All data was analyzed using the Proc Mixed procedures of SAS (SAS Institute Inc., Cary, NC) with calf as the experimental unit. No significant differences were observed for average performance, rectal temperature, or IBR antibody titers with either IN or IM vaccinations ($P > 0.10$). However, serum urea nitrogen and the ratio of serum lysine:arginine increased ($P < 0.05$) for LYS compared to CAS calves. These results suggest that time of administration of a modified-live respiratory viral vaccine can alter feed intake and vaginal temperature in feeder heifers; but no difference in overall performance was observed. Further, these results suggest that supplementing lysine does not alter the response to IBR vaccination or animal performance in neonatal Holstein calves.

Introduction

Bovine respiratory disease (BRD) continues to be the most important health factor in feedlot cattle in North America, accounting for the majority of morbidity and mortality in feedlot cattle (Woolums et al., 2005). Both viral and bacterial pathogens contribute to BRD, therefore both antibiotics and vaccination protocols are used to combat this widespread problem. Although many treatment techniques are available, BRD is still the most prominent health problem in the cattle industry. Economic effects of BRD include decreased ADG, death loss, treatment costs and reduced carcass quality (Smith, 2004; Schneider et al.,

[*]Mention of a trade name, proprietary product, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

2009; Taylor et al., 2010). Calves treated for BRD commonly have lower ADG compared with healthy animals (Fulton et al., 2002; Snowder et al., 2006; Schneider et al., 2009) and infected calves have been reported to return $54.01 less than non-infected calves (Schneider et al., 2009).

Vaccination is a common practice aimed at prevention; although, procedures do not always guarantee prevention. Vaccines are given to promote antibody titers, which should encourage protection of the host against viral and/or microbial pathogens. Vaccination intended for pathogens contributing to BRD are widely available, yet overall immunity acquired after vaccination against respiratory disease pathogens is variable; with Hodgins et al. (2002) reporting only 75% of vaccinated animals are protected from BRD. Further, vaccination in time of stress may reduce immunity gained from vaccination (Kehrli et al., 1999). Vaccine processing strategies differ including vaccine type (intranasal or intramuscular) and timing (arrival or delayed), which have also yielded conflicting results in terms of effectiveness (Hansen et al., 1992; Duff et al., 2000).

Common practices include vaccinating feedlot cattle within 48 h of arrival, or a delayed vaccination, administered 2 to 3 weeks after arrival. Most veterinarians and animal health professionals support vaccinating cattle against BRD on arrival, while some argue that vaccination should be delayed to allow animals to recover from the stresses of shipping. In groups of cattle where morbidity is high, on-arrival vaccination protocol is common (Hansen et al., 1992). However, several studies observed no difference in morbidity when vaccinating receiving cattle on arrival (Bateman, 1998; Duff et al., 2000; Richeson et al., 2008; Richeson et al., 2009) and Martin et al. (1982) reported increased mortality risk when cattle were vaccinated with a respiratory vaccine within the first 14 d of arrival. Increased performance may be an advantage of vaccination. Kreikemeier et al. (1996) reported calves that were vaccinated before weaning and revaccinated on arrival to the feedlot had a greater BW gain than calves receiving a vaccination upon arrival and revaccinating after 21 d at the feedlot. However, timing of vaccination has also shown neutral outcomes concerning performance. Delayed versus on arrival vaccination has been shown to have no effect on animal performance by several groups (Lofgreen et al., 1983; Duff et al., 2000; Richeson et al., 2009). Further, Chirase et al. (2001) observed that calves receiving a saline subcutaneous injection had greater ADG than calves injected subcutaneously with a 7-way clostridial vaccine. Spurlock (1997) reported that repeated vaccination, or repeated immune stimulation, can have a negative effect on growth of an animal. Vaccination timing and its effects on performance may be attributed to overall health state of incoming animals, type of vaccination, and stress animals are subjected to.

Route of vaccine administration may also affect performance in receiving cattle. Respiratory vaccines administered intranasaly (IN) have demonstrated advantages in ADG (Duff et al., 2000). Again, method of vaccination may depend on health state of calves and timing of vaccination. Intranasal vaccines may be beneficial when vaccinating on-arrival because of immediate protection (within 24 hours; Kucera and Beckenhauer, 1978). Kucera and Beckehhauer (1978) reported IN temperature-sensitive IBR vaccine to deliver protection within 24 h. An IM IBR vaccination is also capable of providing protection against a virulent virus within 48 h (Sutton, 1980).

Because of such conflicting results further research is needed in this area. Vaccine type and timing may be especially important in newly received cattle in the feedlot, which are at greatest risk for respiratory disease due to the combination of stress, commingling and pathogen presence. The majority of morbidity during the feeding period occurs within the first three weeks after arrival and 15% to 45% of incoming calves require treatment (Kelly and Janzen, 1986). Cattle may be exposed to pathogens after arriving to a feedlot. For this reason, on arrival vaccination and the rapid onset of immunity may be beneficial (Todd et al., 1971; Sutton, 1980). Although preventative measures are available, morbidity rates due to BRD have continued to increase (Loneragan et al., 2001). Though a pathogenic disease, BRD is aggravated by many external factors. Shipping and processing can increase the risk factors for BRD both immunologically and environmentally. Immunological competence can be compromised due to stress from commingling, nutritional change, shipping distance and weather (Cernicchiaro et al., 2012). A combination of stress and immune challenges may result in morbidity and poor performance (Smith, 2004). Effects of stress due to shipping, environment, and commingling can persist up to 15 d after arrival and continue to negatively impact the immune system and decrease vaccine efficacy (Loerch and Fluharty, 1999; Purdy et. al. 2000). It can be considered that vaccination type and timing can have effects on both morbidity and performance, although reports have been inconsistent as to a superior routine. A lack of consistency of vaccine efficacy and performance may be affected by health state of calves, commingling, susceptibility to pathogens due to stress, and to the dynamic complex of BRD.

Because of external stress contributing to BRD, attention is brought to bovine herpes virus-1 (BHV-1), which is a major contributor to BRD (Yates, 1982). Bovine herpesvirus-1 is unique in that ultimately all animals are exposed and infected to BHV-1 early in life. Following inoculation, animals may exhibit clinical signs or be asymptomatic (Jones et al., 2000). Subsequently, the virus will become latent within nerve cells (Nandi et al., 2009). In stressed animals the virus may exit latency and replicate to produce infectious virus (Pastoret et al., 1982). The replicating virus can induce disease (IBR) and/or spread to susceptible animals. Vaccines are readily available for BHV-1, the goal being to maintain enough immunity to avoid reactivated, clinical disease, although this is not always the case, as sufficient immunity may not last throughout life, and another infection. Further, vaccination does not provide protection against latent infection (Jones et al., 2000). Shortcomings in vaccination encourage additional methods of prevention.

Herpes virus-1 requires arginine to replicate (Griffith et al., 1981). Lysine is a natural inhibitor of

arginine (Maggs et al., 2000); as lysine levels increase, arginine levels will decrease. Lysine as a combatant for herpes replication in felines and humans has been evaluated. In vitro studies of lysine have shown a replication-inhibiting capability of the herpes virus (Maggs et al., 2000). With previous research in other species, lysine has potential to combat a herpes virus infection in stressed cattle. Lysine supplementation may decrease virulence of a herpes virus infection, and/or increase efficacy of current vaccines. A decrease in IBR outbreaks may reduce BRD severity and frequency.

In summary, bovine respiratory disease impacts both herd health and performance, increasing economic expense. Improved measures of processing and vaccination protocol are important components in reducing BRD, promoting cattle health and improving performance. Health management, namely vaccination programs, is essential to successful cattle production. The objective of this research was to investigate vaccination protocol and a potential technique to increase vaccine efficacy. To investigate this, two experiments were conducted, the first focusing on vaccination timing (an on-arrival verses a delayed vaccination) of newly received cattle. The second experiment focused on the effects of supplemental lysine on a modified-live BHV-1 vaccination in calves. Both trials focused on immune measures in response to a modified-live viral vaccination in the form of antibody titer levels and febrile response. Both trials reported performance data. Additionally, in experiment 1 daily intake and eating behavior was constantly monitored using a GrowSafe system.

Materials and Methods

Procedures were reviewed and approved by the Montana State University Agriculture Care and Use Committee, 2012-AA01.

All materials and methods are described by Sharon et al., 2012 and 2013.

Discussion

Cattle are exposed to antigenicity through all stages of life, therefore sufficient, consistent immunity is important in times of stress, such as shipping and processing (Klasing and Barnes, 1988). Common feedlot management practices involve vaccinating cattle for BRD upon arrival with modified-live viral and bacterin-toxoid vaccines. Our first experiment investigated effects of an on arrival vaccination or delayed vaccination in receiving cattle in terms of performance and immune response. The first trial conducted used 36 heifers (initial BW = 265 ± 20 kg) randomly assigned to three treatments including a receiving an on-arrival (d 0; D0) vaccination, a delayed (d 14; D14) vaccination, or no vaccination (CON).

Although vaccination timing differed, no differences were observed among treatments (D0, D14, and CON) for initial BW, final BW, ADG, overall G:F or number of trips to GrowSafe bunk. Eating behavior was affected by vaccination, delaying vaccination altered feeding behavior for approximately 3 d versus altered feed intake for 1 d when the vaccine was administered during the start of the receiving period. Although no difference in performance, a reduction in feed intake in the delayed vaccination group suggests that vaccination may impact DMI. Gandra and Scrimshaw (1961) explained even mild immune taxation such as vaccination has been shown to decrease normal feed intake.

In this experiment, antibody titers did not differ among treatments (D0, D14, and CON) with the exception of BHV-1, which was higher in vaccinated compared to non-vaccinated cattle. Previous vaccination with a modified-live respiratory vaccination and health state of candidate animals was likely to have influenced titer levels and accounted for lack of difference. Bovine herpesvirus-1 titer distinction may have been exemplified due to lack of previous developed immunity. Efficacy of BHV-1 of the modified live vaccine may have been superior within this group of cattle.

Febrile response proved to be affected by vaccination timing, as a delayed vaccination induced an increase in temperature for 3 d post vaccination, an on-arrival vaccination induced a febrile response for 1 d post vaccination. A link between febrile response and nutrition could serve as an explanation for the increased febrile response observed in the D14 compared to the D0 heifers. Further, D14 heifers may have been in a better nutritional state and thus had the ability to generate an amplified immune response in the form of fever. Nutritional status can influence febrile response (Scrimshaw et al. 1991; Scrimshaw and SanGiovanni, 1997). Energy and body tissue lost due to an immune challenge may be restored more rapidly if cattle are in a sound nutritional state. Fever can induce amino acid mobilization from tissues such as muscles, used in cellular immune function (Scrimshaw et al., 1991). Fatty acids may also be utilized during a fever (Long et al., 1977). Basal metabolic rate can increase up to 30% during a febrile response (Cooper et al., 2000). Further, Scrimshaw and SanGiovanni (1997) reported malnourishment results in a reduced ability to produce central immune factors. Dietary energy may play an important role in reducing the incidence and severity of respiratory disease in receiving cattle. As a result, nutritional state of cattle may be important for recovery as well as future performance.

This study addressed an important health area in the cattle industry, receiving cattle. Because this group of cattle is at such high risk for developing BRD, this research as well as future research is important for improving processing practices and encouraging herd health.

Immunity in cattle prior to the receiving period may be important in reducing BRD vulnerability. Alternative measures for reducing BRD is an area of growing interest due to issues such as lack of vaccine efficacy and concerns of bacterial resistance. Our second experiment investigated combating IBR by supplementing lysine, potentially improving vaccine efficacy. In experiment 2, 64 neonatal Holstein calves allocated to three treatments including supplemental lysine (LYS) supplemental casein (CAS) or neither supplement (CON). Two groups of calves received an intramuscular (IM) modified-live IBR vaccination and two groups received an

intranasal (IN) modified-live IBR vaccination. Febrile response and antibody titer values were used to measure immune response. No differences were observed for initial BW, final BW, ADG, DMI or overall G:F across treatments (LYS, CAS, and CON). These results are similar to Aubry et al. (2001) reporting no difference in daily gain among vaccinated and non-vaccinated Holstein calves. No differences were observed among treatments (LYS, CAS, and CON) in vaccinated calves for IN or IM, for rectal temperature. Similarly, no difference in IBR antibody titer levels was observed. In young cattle, maternal antibodies may play a significant role in vaccination efficacy in young animals (McGuire et al., 1976; Lamiare et al., 2000). These maternal antibodies can persist in calves for up to 230 d after birth (McGuire et al., 1976). Menanteau-Horta et al. (1985) found calves with maternal antibodies still present had reduced active immunity formation from an intramuscular IBR modified-live viral vaccination. Lack of febrile response as well as antibody titer response may have been a result of present maternal antibodies. These factors and results from this study make timing of vaccination in young calves is an important consideration for developing immunity.

Although no performance or immunity differences among treatments, serum urea nitrogen and lysine:arginine ratio was greater for LYS compared to CAS calves. These results suggest that supplemental lysine may manipulate nitrogen metabolism in neonatal calves.

While immune measures were not affected, lysine may contribute nutritionally. Nutritional status is important for all bodily functions including immunity (Chandra, 1997; Lochmiller and Deerenberg, 2000). Cattle exposed to stress and/or pathogens require adequate nutrition to maintain health (Cole, 1996). This topic is specifically relevant in receiving cattle, which may be greatly affected by nutrition, as they will be exposed to stress and pathogens as well as likely reductions in nutritional consumption after arrival. Receiving cattle may have lower DMI due to factors such as herd hierarchy and stress, resulting in a depressed nutritional state (Gibb et al., 2000). Sick cattle often have reduced feed intake, accentuating reduced nutritional status (Lofgreen et al., 1983). Cattle with poor nutritional reserves may be unable to withstand a pathogenic infection, leading to BRD. Further, additional dietary energy has shown evidence of improved immune cell function (Stabel et al., 2003).

These trials as well as previous research demonstrate vaccination timing as well as nutritional status may be influential to cattle health, both early in life and during the feedlot-receiving period. This information may be used the timing and type of vaccinations used in previously vaccinated, healthy receiving cattle or young calves.

Implications and Future Research

Vaccinating cattle with a modified live respiratory vaccine will increase body temperature and alter feed intake for a short duration; thus, managers can use these data when determining which vaccination protocols to utilize on various groups of receiving cattle. It's important to note that the short durations of febrile response and decreased feed intake observed in the current study didn't result in overall losses in ADG or gain efficiency. Further, these results suggest that supplemental lysine will not alter febrile response or IBR antibody titer levels after a modified-live respiratory vaccine but will alter SUN and serum lysine:arginine ratios in neonatal calves.

Future research to elaborate these findings would include investigation of lysine supplementation and its relationship to a bovine herpesvirus-1 challenge. Lysine levels may affect a live virus more so than a modified-live vaccination. Lysine may still have the potential to reduce severity of a BHV-1 infection. A viral challenge may elucidate effects of lysine immunologically. Investigation of intramuscular or intransal boosters may be helpful in understanding response in young calves. Also, monitoring immune measures throughout life would be significant in understanding immune response to vaccinations. A better understanding of cattle immunity through life may influence current vaccination protocols and increase efficacy. Specifically, monitoring cattle vaccinated on-arrival or after a delayed period through slaughter may elucidate any differences in performance, health or carcass characteristics. This research created a foundation for further investigation of vaccine protocols and alternative techniques for improved vaccine efficacy.

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EVALUATION OF THE NOVEL FEEDSTUFF, LIPID EXTRACTED ALGAE, FOR RUMINANT ANIMALS IN FORAGE AND CONCENTRATE DIETS

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ABSTRACT: Two experiments were conducted to evaluate lipid extracted algae (**LEA**), the co-product generated by extracting oil from microalgae grown for biofuel production on feed intake and nutrient digestibility. Experiment 1 compared LEA to soybean meal (SBM) in a forage diet. Fifteen lambs (43 ± 1.4 kg BW) fitted with ruminal cannulas were used in a completely randomized design (CRD). Experimental diets included: 1) sorghum-sudan hay (CP 8.27%; NDF 51.66%, DM basis; **HAY**), 2) sorghum-sudan hay plus LEA (CP 14.06%; NDF 43.37%, DM basis; **ALGAE**), and 3) sorghum-sudan hay plus SBM (CP 10.20%; NDF 50.05%, DM basis; **SOY**). Total tract CP digestibility was lowest ($P < 0.01$) for ALGAE while SOY and HAY did not differ. Inclusion of SBM increased ($P < 0.01$) ruminal ammonia by 57.4% and 56.0% compared with ALGAE and HAY, respectively. Ruminal acetate was greatest ($P = 0.04$) for HAY, lowest for SOY, and ALGAE did not differ from HAY and SOY. Ruminal propionate was greatest ($P = 0.04$) for ALGAE, which differed from HAY, while SOY was similar to ALGAE and HAY. Acetate:Propionate ratio was lowest ($P = 0.03$) for ALGAE. Adding LEA to a forage-based diet resulted in increased propionate production, while OM and NDF digestibility were comparable to SOY. However, the inclusion of LEA in a forage diet resulted in the lowest ruminal ammonia and total tract CP digestibility indicating that LEA is less degradable than SBM therefore hypothesis is invalid. Experiment 2 hypothesized that an isonitrogenous addition of LEA in a high-concentrate diet would yield results similar to that of dried distillers grains with solubles (DDGS). Fifteen lambs (46 ± 7.1 kg BW) fitted with ruminal and duodenal cannulas were used in a CRD. Treatments were: 1) Lipid Extracted Algae (CP 16.3%; NDF 23.4%, DM basis; n = 8; **ALGAE**) or 2) DDGS (CP 15.7%; NDF 22.3%, DM basis; n = 7; **DGS**) added to a concentrate-based diet. Ruminal and total tract OM digestibility were lower for ALGAE compared to DGS. Ruminal N digestibility was unaffected by treatment ($P > 0.10$), however N digestion in the total tract was lower ($P < 0.01$) for ALGAE than DGS. Total tract NDF digestibility was greater ($P = 0.05$) for DGS than ALGAE. Microbial efficiency tended ($P = 0.09$) to increase for LEA compared to DGS. Ruminal pH, NH_3, and VFA concentrations did not differ by treatment ($P \geq 0.34$). These data imply that CP in LEA may be of similar ruminal solubility to CP in DDGS but lower than SBM.

Key Words: Biofuels, Sheep, Digestibility

Introduction

Biofuels are currently classified into one of two generations. First generation biofuels originate from food crops, while second generation biofuels are derived from non-food sources such as cellulosic biomass, microalgae, and agricultural wastes (Patil et al., 2008). Advanced biofuels are derived from renewable biomass including cellulosic ethanol and microalgae, that create no more than half of the greenhouse gas emissions of the fuel they replace (Cortes-Caminero, 2010).

Microalgae are photosynthetic cell factories that convert sunlight and carbon dioxide to biomass which includes lipids, proteins and carbohydrates (Chisti, 2007). According to DOE (2010), microalgae has advantages as a biofuel feedstock including: 1) high biomass yield per unit cultivation; 2) minimal competition for land used in traditional agriculture; 3) cultivation uses various water sources such as waste, saline, and produced water; 4) cultivation can utilize CO_2 emissions; and 5) biomass from algae produces both fuel and valuable co-products.

Producing fuels from algal cells begins with extraction methods, where the lipid is separated from the algal cell. The lipid is the refined to produce transportation and aviation biofuels. The remaining portion termed lipid extracted algae (LEA) and is made up of carbohydrates, protein, and un-extracted lipids. Our laboratory nutrient analysis of LEA, from *Nannochloropsis sp.*, reveals that LEA ranges in crude protein from 17 to 35% (unpublished data).

Two experiments were designed to evaluate the intake and digestibility of LEA when included in sheep diets. In Experiment 1, we hypothesized that an isonitrogenous addition of LEA to a forage diet would yield similar feed intake and diet digestibility when compared to soybean meal (SBM). Experiment 2 tested the hypothesis that an isonitrogenous addition of LEA to a high concentrate diet would yield similar feed intake and diet digestibility when compared to dried distillers grains with solubles (DDGS).

Materials and Methods

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Fifteen crossbred wether lambs (43 ± 1.4 kg BW) were fitted with a J-style duodenal and ruminal cannula. Lambs were individually housed in a temperature controlled room within 1.2 m x 2.3 m pens with concrete

floors. Pens were bedded with wood shavings. Lambs were randomly assigned to pen and treatments were randomly assigned to individual pens.

Diets. *Nannochloropsis* sp. LEA used in these studies was purchased as a solvent extracted, dried, granular ingredient (CP 25.9%; NDF 24.7%; EE 15.3%; Ash 9.1%, DM basis). Animals were allowed *ad libitum* access to water and treatments were delivered twice daily, approximately 12 h apart, at 110% of previous 3 d DMI.

In Experiment 1, lambs were assigned to 1 of 3 dietary treatments in a completely randomized design. Experimental diets included: 1) sorghum-sudan hay (CP 8.27%; NDF 51.66%, DM basis; **HAY**), 2) HAY plus LEA (CP 14.06%; NDF 43.37%, DM basis; **ALGAE**), and 3) HAY plus SBM (CP 10.20%; NDF 50.05%, DM basis; **SOY**).

In Experiment 2, lambs were assigned to 1 of 2 dietary treatments in a completely randomized design. Treatments were: 1) Lipid Extracted Algae (CP 16.3%; NDF 23.4%, DM basis; n = 8; **ALGAE**) or 2) DDGS (CP 15.7%; NDF 22.3%, DM basis; n = 7; **DGS**) added to a concentrate-based diet.

Sampling and Laboratory Analysis. The experimental period for each study was 15-d, with 10-d allotted for diet adaptation and 5-d of intensive sampling. Feed, orts, duodenal (Experiment 2 only) and fecal samples were collected for analysis. Ruminal fluid samples were collected for VFA, passage rate determination, ruminal pH, and NH_3 analysis. Laboratory analyses were performed as described in Beckman et al. (2013).

Calculations and Statistical Analysis. Digestibility and nutrient flow was calculated as described in Scholljegerdes et al. (2004). All data were analyzed as a completely random design using the MIXED procedure of SAS (version 9.2; SAS Inst. Inc., Cary, NC) with repeated measures for ruminal VFA and NH_3. Animal was the experimental unit and treatment was the random variable. The model included treatment, day, and treatment × day interactions. Using Akaike's information criterion, compound symmetry was the covariance structure. Means were calculated using LSMEANS. Treatment effects were considered significant at a $P \leq 0.05$ and as a tendency if $P > 0.05$ and ≤ 0.10. When *F*-tests were significant, mean separations were performed using a pair-wise comparison (PDIFF). There were no treatment x day interactions ($P \geq 0.24$), therefore main effects are presented.

Results and Discussion

Experiment 1. Data from Experiment 1 are presented in Table 1. The addition of LEA or SBM to a forage diet did not affect OM, CP or NDF ($P \geq 0.12$) intake. Total tract digestibility of OM and NDF were not affected ($P \geq 0.12$). These data differ from Guthrie and Wagner (1988) who demonstrated that increasing levels of SBM (0, 121, 241, 362, 603 g/d) added to a forage diet increased DMI in beef heifers. Bodine et al. (2000) evaluated the effects of supplementing corn and SBM to beef steers consuming prairie hay and reported that with increasing level of SBM supplementation (0, 33, 66, 100% of DIP requirement) there was an increase in total OM intake. One possible explanation is that the CP in the hay used by Guthrie and Wagner (1988) was 5% CP and in Bodine et al. (2000) was 6.1%. According to Mathis et al. (2000), when the CP in forage is above 7% (with a range of 6-8%), protein supplementation does not improve digestibility.

Total tract digestibility of CP was similar for HAY and SOY treatments, while ALGAE reduced ($P < 0.01$) CP digestibility by 7.8% and 11.2% compared to HAY and SOY, respectively. These data suggest that CP in LEA, may not be as digestible as CP in SBM or HAY. These observations are further supported by ruminal NH_3 values observed in this experiment. Specifically, ruminal NH_3 was greatest ($P < 0.01$) for SOY, while ALGAE and HAY did not differ (6.61 vs. 3.66 and 3.72 ± 0.56 mM, respectively). Soybean meal is 66% RDP (NRC, 2000), which is considered a high source of soluble protein. A linear increase in ruminal NH_3 was seen by Bodine et al. (2000), when increasing levels of SBM were included with a diet of prairie hay and supplemental corn. According to Satter and Slyter (1974) ruminal NH_3 levels should range 1.2 to 2.9 mM to support ruminal bacterial growth. Ruminal NH_3 levels observed in our study were well within this range, therefore, N was not limiting in the rumen and likely was not the reason for a reduction in total tract CP digestibility.

Ruminal fluid outflow rate for HAY was 66% higher than for SOY and ALGAE ($P = 0.05$). It is not clear as to why ruminal outflow differed to this extent, however, one could speculate that because HAY had a greater proportion of the diet as Sorghum-sudan hay (66.5% of DM), salivary production was increased, which in turn increased fluid entering the rumen and ultimately fluid outflow (Jacques et al., 1989).

Ruminal pH did not differ by treatment ($P = 0.96$). Total VFA and butyrate were similar across treatments ($P \geq 0.14$). Ruminal acetate was lowest ($P = 0.04$) for SOY, while HAY and ALGAE were similar. Ruminal propionate was greatest ($P = 0.04$) for ALGAE, however SOY was similar to both ALGAE and HAY. This resulted in a treatment difference ($P = 0.03$) for the acetate to propionate ratio which was lowest for ALGAE versus HAY and SOY was similar to both other treatments. Because VFA represent the main supply of metabolizable energy for ruminants (Van Soest, 1982), a reduction in total VFA production would be energetically unfavorable for the nutrition of the animal (Busquet et al., 2006). These data indicate that the inclusion of LEA, while not increasing total VFA production could cause a shift proportion of individual VFA, particularly propionate. Similar results have been seen when supplementing sources of RDP to forage based diets, which was attributed to a greater level of starch in the supplement fed (Hannah et al., 1991). This may suggest that the LEA in the ALGAE treatment has a higher fraction of soluble carbohydrate than originally expected, and would account for the shift in individual VFA.

Experiment 2. Data from Experiment 2 are presented in Table 2. The inclusion of LEA or DDGS in a concentrate diet did not affect ($P > 0.21$) OM, CP or NDF intake. Similar OM intake of ALGAE and DGS diets implies that when included in a high-concentrate diet, LEA may be of similar palatability to DDGS. The work of

Schauer et al. (2008) demonstrated that substituting DDGS for SBM and barley in a total mixed ration, when fed to lambs, increased feed intake with greater DDGS inclusion level. Lambs in this trial as well as in a previous trial comparing SBM to LEA in a forage diet (Beckman et al., 2012) showed similar intakes to both SBM and DDGS, suggesting that the palatability of LEA, when included in a total mixed ration may be favorable. Results from the current study show that lambs consuming 15% DDGS had similar intake to lambs consuming 19% LEA, offering potential starting values for further work investigating optimal inclusion of LEA in a high-concentrate diet.

Ruminal and total tract digestibility of OM was lower for ALGAE versus DGS ($P = 0.05$ and $P < 0.01$, respectively). The lower tract digestibility of OM was unaffected ($P = 0.93$) by treatment. There was no difference in OM intake between treatments, which according to Galyean and Owens (1991) can affect digestibility along with passage rate. Differences in the OM digestion for the rumen and total tract may be due to size of particulate matter of the LEA included in the diet causing increased passage rate as noted in Firkins et al. (1985). Total tract digestibility of NDF was increased for DGS over ALGAE ($P = 0.05$), suggesting a higher level of insoluble fiber in LEA over DDGS.

Ruminal digestibility of CP showed no significant effect of treatment ($P = 0.11$), however ALGAE had a tendency for lower ruminal degradability compared to DGS. Breakdown of CP in the total tract was lower ($P < 0.01$) for ALGAE than for DGS. These data may indicate that the protein in LEA is similar to DDGS in rumen degradability, but continues to be less available to the host animal in the lower tract, specifically the small intestine. Firkins et al. (1985) compared SBM, wet and dry corn gluten feed and dried distillers grains in concentrate diets to feedlot cattle. The steers consuming dried distillers grains gained weight faster and in a more efficient manner than did steers consuming the other treatments. Firkins attributed this to the RUP levels of the dried distillers grain, which allowed the small intestine an increased influx of amino acids which escaped microbial fermentation. This supporting data shows the value of the RUP in dried distillers grains and may indicate similar value of RUP in LEA. A tendency ($P = 0.09$) for ALGAE to increase microbial efficiency over DGS was noted. Microbial efficiency is the amount of microbial N that is produced per kg OM that is truly fermented. The mechanism behind this tendency is curious because there was a significant decrease ($P = 0.05$) in OM fermented in the rumen for the ALGAE over DGS treatments, yet ALGAE maintain a higher microbial efficiency. Leupp et al. (2009) saw an increase in microbial efficiency with increasing level of DDGS fed to steers in a high concentrate diet, but attributed this to a decrease in the OM fermentation of the rumen with increasing levels of DDGS. Additional work in this area is needed to characterize this response.

Ruminal pH and ruminal NH_3 did not differ by treatment ($P \geq 0.82$). Ruminal NH_3 levels are an indication of protein breakdown in the rumen, as protein is the most abundant source of N in the ruminant diet (Wallace et al., 1997). The NRC (2000) reports that the crude protein in distillers grains with solubles is 27% digestible intake protein, suggesting that it is a good source of RUP. The similarity of NH_3 level for ALGAE and DGS suggests that the novel protein LEA may be a good source of RUP.

Total VFA, acetate, propionate and butyrate, along with the acetate:propionate did not differ by treatment ($P \geq 0.34$). Similar values for VFA production between ALGAE and DGS treatments indicate that LEA and DDGS included in a concentrate diet behave comparably in the production of VFA. Leupp et al. (2009) documented that feeding increasing levels of DDGS to steers consuming seventy percent concentrate diets showed a linear reduction in total VFA with increasing level of DDGS. Also reported in this study was a decrease in molar proportion of acetate and increase in molar proportion of propionate with increasing DDGS level in the diet. Opportunities for further work continue to point towards investigating the inclusion level of LEA, much the same way Leupp et al. (2009) studied here. The potential to increase propionate production and decrease acetate production is of interest to ruminant nutritionists, as propionate is a glucogenic precursor, however, one must also consider that Leupp et al. (2009) saw a decrease in total VFA production which Busquet et al. (2006) warns is energetically unfavorable for the nutrition of the animal.

Implications and Future Work

These results imply that the addition of lipid extracted algae to a forage diet will increase propionate, an important glucogenic precursor. Furthermore, similar organic matter intake indicates that lipid extracted algae may be as palatable as soybean meal and dried distillers grains with solubles. Based on the results of Experiment 1 and 2 we believe that the inclusion of lipid extracted algae in ruminants diets is feasible. However, there are some issues upstream processes may influence and should be addressed, such as the possibility of a higher proportion of insoluble fiber in the algal biofuel co-product. Also, the availability of protein in the small intestine will largely affect the projected value of algal biofuel co-products. Similar ruminal fermentation characteristics are encouraging and offer researchers an opportunity to delve into more concentrated lipid extracted algae studies. These studies would evaluate inclusion level on fermentation characteristics, the possibility of shifting proportions of individual VFA, and looking for changes in total VFA production. These studies would evaluate inclusion level on fermentation characteristics, the possibility of shifting proportions of individual VFA, and looking for changes in total VFA production.

When considering the future of LEA as a feed ingredient in the livestock industry, there are many other avenues for future research work. Current algal biofuel industry practices do not allow for marketing of singular uniform product that is created following a rigid set of production guidelines. Rather, LEA is generated from various species of microalgae, grown by differing production practices, with lipids extracted in several differing procedures. This is a driving force behind the need for additional research. Currently, given the early stages of

development of this industry, limited amount of LEA is available from the variety of cultivation and processing practices. Usually there is enough material to perform nutrient analysis. However, this does not allow the animal scientist to make deductions about the site and extent of digestion of LEA in the gastrointestinal tract, nor does it provide an indication of palatability or animal performance. In addition to protein, fiber and lipid analysis, complete mineral, heavy metal and amino acid analysis is also needed. Mineral and heavy metal analysis are necessary, due to the variation in water sources and extraction methodologies. If specific mineral or heavy metal levels in LEA exceed the maximum tolerable level of a species, it will limit inclusion rates and decrease the value of LEA. A complete amino acid profile of LEA, particularly given its potential RUP level, may give added value to LEA if it were to be a source of amino acids commonly limiting in ruminant diets.

The purpose behind the research of LEA included in this paper is part of a large scale push towards assisting the United States in becoming energetically independent and the need for evaluation of co-product markets made these live-animal studies possible. Any continued work in these areas, while they would benefit the animal production industry, will first serve as a guide to algal biofuel producers in their quest to find appropriate co-product markets.

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Table 1. Influence of adding lipid extracted algae or soybean meal to sorghum-sudan hay.

	Treatments[1]				
Item	HAY	ALGAE	SOY	SEM[2]	*P*-value
OM Intake, g/Kg BW	12.5	12.6	12.9	2.167	0.99
CP Intake, g/Kg BW	1.0	1.7	1.2	0.207	0.12
NDF Intake, g/Kg BW	7.3	5.6	6.9	1.077	0.53
Total Tract Digestibility					
OM	62.5	55.4	66.4	3.524	0.12
CP, % of OM	82.1[a]	75.9[b]	84.9[a]	1.177	< 0.01
NDF, % of OM	81.6	79.6	81.0	2.771	0.88
Ruminal pH	6.8	6.8	6.8	0.065	0.96
Ruminal NH_3, mM	3.7[a]	3.7[a]	6.6[b]	0.560	< 0.01
Ruminal Volume, L	8.6	5.8	7.5	1.017	0.19
Ruminal Fluid Outflow, L/h	0.5[a]	0.3[b]	0.3[b]	0.061	< 0.05
Ruminal Fluid Dilution, %/h	6.2	5.5	3.9	0.798	0.16
Ruminal total VFA, mM	58.8	66.6	67.8	7.603	0.67
Ruminal VFA, mol/100 mol					
Acetate	74.1[a]	71.6[ab]	69.1[b]	1.245	0.04
Propionate	14.8[a]	19.9[b]	17.8[ab]	1.233	0.04
Butyrate	9.2	6.7	8.2	0.837	0.14
Acetate:propionate	5.0[a]	3.7[b]	4.1[ab]	0.307	0.03

[1]Treatments: **HAY**= sorghum-sudan hay (6.4% CP and 62.3% NDF; DM basis) with no supplemental protein; **ALGAE**= HAY plus lipid extracted algae; **SOY**= HAY plus soybean meal.
[2]n=5
[ab]values within rows with differing superscripts differ $P \leq 0.05$ between treatments.

Table 2. Influence of adding lipid extracted algae or dried distillers grains to concentrate diets.

	Treatments[1]			
Item	ALGAE	DGS	SEM[2]	*P*-value
Intake, g/Kg BW				
OM Intake	21.8	18.7	2.38	0.21
CP Intake	3.5	3.0	0.36	0.21
NDF Intake	0.6	0.5	0.06	0.21
Digestibility				
OM Digestibility				
Ruminal, % entering	78.6	83.6	1.77	0.05
Lower Tract, % entering	37.1	36.6	4.43	0.93
Total Tract, % intake	81.0	86.8	1.38	< 0.01
CP Digestibility				
Ruminal, % entering	82.3	84.9	1.14	0.11
Total Tract, % intake	76.0	86.1	1.49	< 0.01
NDF Digestibility				
Total Tract, % intake	70.5	76.6	2.19	0.05
Microbial efficiency, g/kg of OM fermented	6.7	4.0	1.15	0.09
Ruminal pH	6.3	6.3	0.17	0.82
Ruminal NH_3, mM	6.3	6.5	1.08	0.89
Ruminal total VFA, mM	76.6	70.4	12.90	0.73
Ruminal VFA, mol/100 mol				
Acetate	48.9	46.9	7.78	0.85
Propionate	17.0	14.1	4.80	0.67
Butyrate	9.3	8.8	1.90	0.84
Acetate:propionate	5.4	4.1	0.91	0.34

[1]Treatments: **ALGAE**= concentrate diet with protein supplement from lipid extracted algae; **DGS** = concentrate diet with protein supplement from dried distillers grains with solubles
[2] **ALGAE** (n = 8), **DGS** (n = 7)

GRADUATE STUDENT PAPER COMPETITION

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EFFECTS OF FLUNIXIN MEGLUMINE ADMINISTRATION ON ACUTE-PHASE AND PERFORMANCE RESPONSES OF TRANSPORTED FEEDER CATTLE

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ABSTRACT: The objective of this experiment was to evaluate the effects of flunixin meglumine administration on physiological and performance responses of transported cattle during feedlot receiving. Forty-five Angus × Hereford steers were ranked by BW on d 0, and assigned to 1 of 3 treatments: 1) transport for 1,280 km in a commercial livestock trailer and administration of flunixin meglumine (1.1 mg/kg of BW; i.v.) at loading (d 0) and unloading (d 1; **FM**); 2) transport for 1,280 km in a commercial livestock trailer and administration of 0.9% saline (0.022 mL/kg of BW; i.v.) at loading (d 0) and unloading (d 1; **TRANS**), or 3) no transport and administration of 0.9% saline (0.022 mL/kg of BW; i.v.) concurrently with loading (d 0) and unloading (d 1) of FM and TRANS cohorts (**CON**). On d 1, steers were ranked by BW within each treatment and assigned to 15 feedlot pens. Full BW was recorded prior to (d -1 and 0) treatment application and at the end of experiment (d 28 and 29) for ADG calculation. Total DMI was evaluated daily from d 1 to 28. Blood samples were collected on d 0, 1, 4, 7, 10, 14, 21, and 28. Body weight shrink from d 0 to d 1 was reduced ($P < 0.01$) and mean ADG was greater ($P < 0.04$) in CON vs. FM and TRANS, but similar ($P = 0.94$ and $P = 0.69$, respectively) between TRANS and FM. No treatment effects were detected on DMI, but CON had greater G:F vs. TRANS and FM ($P < 0.08$). Mean plasma cortisol tended to be greater ($P < 0.09$) in TRANS vs. FM and CON, but was similar ($P = 0.87$) between CON and FM. Plasma NEFA were greater ($P < 0.02$) for TRANS and FM vs. CON on d 1, and greater ($P < 0.04$) for FM vs. TRANS and CON on d 4. Plasma ceruloplasmin concentrations were greater ($P < 0.03$) for TRANS vs. CON on d 1, 4, and 7, greater ($P < 0.05$) for TRANS vs. FM on d 4 and 7, and greater ($P < 0.04$) for FM vs. CON on d 1 and 4. Plasma haptoglobin concentrations were greater ($P < 0.01$) for TRANS vs. CON and FM on d 1 and 4, and greater ($P < 0.05$) for FM vs. CON on d 1 and 4. In conclusion, flunixin meglumine reduced the cortisol and acute-phase protein responses elicited by road transport, but did not improve receiving performance of feeder cattle.

Keywords: Acute-phase proteins, cattle, flunixin meglumine, transport

Introduction

Road transport is one of the most stressful events encountered by feeder cattle during their productive lives (Arthington et al., 2005). Upon long transportation periods and feedlot arrival, cattle experience inflammatory and acute-phase responses (Cooke et al., 2011) that often lead to impaired health and productivity during feedlot receiving (Araujo et al., 2010). Accordingly, management strategies that lessen the magnitude of the acute-phase protein response during feedlot receiving have been shown to improve productivity of transported cattle (Arthington et al., 2008).

One alternative to reduce the acute-phase protein response elicited by road transport is to provide anti-inflammatory agents to cattle. As an example, feeder steers supplemented with linolenic acid had reduced acute-phase protein response and improved performance during feedlot receiving compared with non-supplemented cohorts (Cooke et al., 2011; Cappellozza et al., 2012). Another alternative includes administration of flunixin meglumine, a non-steroidal anti-inflammatory drug, when feeder cattle are processed for transport and feedlot arrival. Accordingly, Merril et al. (2007) reported that flunixin meglumine administration prior to road transport alleviated transport-elicited inflammatory reactions in gestating beef cows. Based on this rationale, we hypothesized that administration of flunixin meglumine prior to transport and at feedlot entry alleviates the acute-phase protein response and improves performance of feeder cattle during feedlot receiving. Hence, the objective of this experiment was to evaluate the effects of flunixin meglumine administration on circulating concentrations of cortisol, NEFA, acute-phase proteins, and feedlot receiving performance of transported cattle.

Materials and Methods

Animals and diets. Forty-five Angus × Hereford steers, weaned 35 d prior to the beginning of the experiment, were ranked by initial BW (228 ± 3 kg; initial age 206 ± 3 d) and assigned to 1 of 3 treatments on d 0: 1) transport for 1,280 km (approximately 24 h) in a commercial livestock trailer and administration of flunixin meglumine (Banamine®; Merck Animal Health; Summit, NJ; 1.1 mg/kg of BW; i.v.) at loading (d 0) and unloading (d 1; **FM**); 2) transport for 1,280 km (approximately 24 h) in a commercial livestock trailer and administration of 0.9% saline (0.022 mL/kg of BW; i.v.) at loading (d 0) and unloading (d 1; **TRANS**), or 3) no transport and administration of 0.9% saline (0.022 mL/kg of BW; i.v.) concurrently with loading (d 0) and unloading (d 1) of FM and TRANS cohorts (**CON**). The flunixin meglumine dose used herein was based on the daily limit indicated by the manufacturer (2 injections of 1.1 mg/kg of BW within 24 h), whereas the CON treatment was included as a non-transport positive control for physiological and performance measurements.

From d -15 to 0, steers were maintained in a single drylot pen (50 × 100 m) and fed alfalfa-grass hay ad libitum and 2.3 kg/animal daily (DM basis) of a concentrate containing (as-fed basis) 84% cracked corn, 14% soybean meal, and 2% mineral mix. Steers assigned to FM and TRANS were transported at the same time and in the same double-deck commercial livestock trailer, while CON steers remained in the same drylot pen (50 × 100 m) with ad libitum access to alfalfa-grass hay and 2.3 kg/animal (DM basis) of the aforementioned concentrate. Immediately upon arrival of FM and TRANS cattle and treatment administration on d 1, steers were ranked by BW within each treatment and assigned to 15 feedlot pens (5 pens/treatment; 3 steers/pen; 7 × 15 m) for a 28-d feedlot receiving. During feedlot receiving, all pens were fed alfalfa-grass hay ad libitum and 2.3 kg/animal daily (DM basis) of the aforementioned corn-based concentrate, which was offered separately from hay at 0800 h. Water was offered for ad libitum consumption from d -15 to 28, except to FM and TRANS cattle during transport.

All cattle were vaccinated against clostridial diseases (Clostrishield 7; Novartis Animal Health; Bucyrus, KS) and bovine virus diarrhea complex (Virashield 6 + Somnus; Novartis Animal Health) at approximately 30 d of age. At weaning (d -35), cattle were vaccinated against clostridial diseases and *Mannheimia haemolytica* (One Shot Ultra 7; Pfizer Animal Health; New York, NY), infectious bovine rhinotracheitis, bovine viral diarrhea complex, and pneumonia (Bovi-Shield Gold 5 and TSV-2; Pfizer Animal Health), and administered an anthelmintic (Dectomax; Pfizer Animal Health). On d 0, 2 steers assigned to CON and 1 steer assigned to TRANS presented symptoms of pneumonia and required medication (0.1 mL/kg of BW of 300 PRO LA, Norbrook Inc.; Lenexa, KS); therefore, these steers were removed from the experiment. No other incidences of morbidity or mortality were observed from d 0 to d 28.

Sampling. Individual full BW was recorded and averaged over 2 consecutive days prior to treatment application (d -1 and 0) and at the end of the experiment (d 28 and 29) for ADG calculation. Individual BW was also collected on d 1, immediately after treatment application, to evaluate BW shrink as percentage change from the average BW recorded on d -1 and 0. Concentrate, hay, and total DMI were evaluated daily from d 1 to 28 from each pen by collecting and weighing orts daily. Samples of the offered and non-consumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of animals within each pen, and expressed as kg per animal/d. Total BW gain and DMI of each pen from d 1 to 28 were used for feedlot receiving G:F calculation.

Blood analysis. Blood samples were collected on d 0 and 1 immediately before treatment application, and on d 4, 7, 10, 14, 21, and 28, via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with or without 158 USP units of freeze-dried sodium heparin for plasma and serum collection, respectively. Blood samples were collected prior to concentrate feeding, except for d 0 when FM and TRANS cattle were transported after blood collection. All blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection. Plasma concentrations of cortisol were determined in samples collected from d 0 to d 10 using a bovine-specific commercial ELISA kit (Endocrine Technologies Inc., Newark, CA). Plasma concentrations of ceruloplasmin and haptoglobin were determined in all samples according to colorimetric procedures previously described (Demetriou et al., 1974; Cooke and Arthington, 2012). Serum concentrations of NEFA were determined in samples collected from d 0 to d 10 using a colorimetric commercial kit (HR Series NEFA – 2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA). The intra- and inter-assay CV were, respectively, 9.1 and 9.8% for cortisol, 6.7 and 7.3% for NEFA, 8.9 and 10.5% for ceruloplasmin, and 7.1 and 11.6% for haptoglobin.

Statistical analysis. Data were analyzed using animal as the experimental unit, given that treatments were individually administered to steers, with the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for BW shrink from d 0 to d 1 and ADG contained the effects of treatment. Data were analyzed using animal(treatment × pen) as random variable. The model statement used for DMI and G:F contained the effects of treatment, as well as day and the treatment × day interaction for DMI only. Data were analyzed using pen(treatment) as the random variable. The model statement used for blood variables contained the effects of treatment, day, the treatment × day interaction, and values obtained on d 0 as covariate. Data were analyzed using animal(treatment × pen) as the random variable. The specified term for the repeated statements was day, pen(treatment) or animal(treatment × pen) as subject for DMI or blood variables, respectively, and the covariance structure utilized was based on the Akaike information criterion. Results are reported as least square means, as well as covariately adjusted least square means for blood variables, and were separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main effects if no interactions were significant, or according to the highest-order interaction detected.

Results and Discussion

A treatment effect was detected ($P < 0.01$) for BW shrink from d 0 to 1. As expected, BW shrink was greater ($P < 0.01$) for both TRANS and FM compared with CON steers, but similar between TRANS and FM steers (Table 1). Supporting these findings, previous research from our group reported equivalent BW shrink rates in feeder cattle exposed to the same transportation schedule adopted herein (Marques et al., 2012). A treatment effect was also detected ($P < 0.01$) for ADG (Table 1). Steers assigned to CON had greater ADG compared with TRANS ($P = 0.04$) and FM ($P = 0.01$) cohorts, whereas ADG was similar between ($P = 0.69$) TRANS and FM steers. However, treatment effects

detected on ADG were not sufficient to impact ($P = 0.37$) cattle BW at the end of the experimental period (Table 1). No treatment effects were detected ($P \geq 0.94$) on hay, concentrate, and total DMI (Table 1). Nevertheless, a treatment effect was detected ($P = 0.02$) for G:F because CON had greater G:F compared with FM ($P = 0.02$) and tended to have greater G:F compared with TRANS steers ($P = 0.08$), whereas G:F was similar ($P = 0.68$) between TRANS and FM steers (Table 1). Hence, FM steers experienced a similar decrease in feedlot receiving performance compared with TRANS cohorts, indicating that flunixin meglumine administration failed to reduce the performance losses caused by road transport.

Table 1. Feedlot receiving performance (28 d) of steers transported for 1,280 km and administered flunixin meglumine (**FM**) or 0.9% saline (**TRANS**) at loading (d 0) and upon arrival (d 1), or non-transported steers administered 0.9% saline (**CON**)[1].

Item	CON	FM	TRANS	SEM	*P* =
BW, kg					
Initial	229	229	227	5	0.93
Final	268	257	255	6	0.27
Shrink, %	0.46[a]	8.85[b]	8.89[b]	0.43	< 0.01
ADG, kg/d	1.18[a]	0.99[b]	1.02[b]	0.05	0.04
DMI, kg/d					
Hay	4.88	4.86	4.95	0.18	0.94
Concentrate	2.21	2.22	2.21	0.05	0.98
Total	7.09	7.08	7.16	0.21	0.96
G:F, g/kg	171[a]	146[b]	149[ab]	6	0.02

[1] Within rows, values with different superscripts differ ($P < 0.05$).

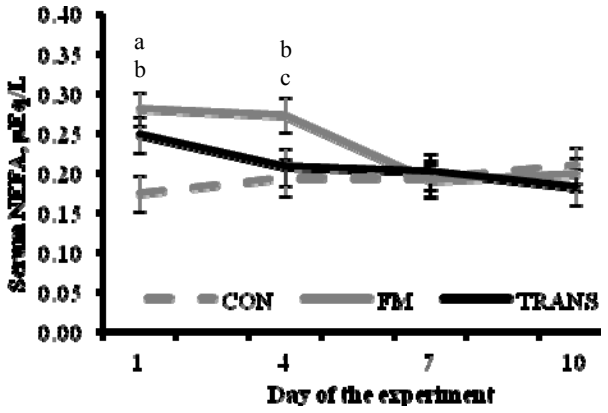

Figure 1. Serum NEFA concentration in steers transported for 1,280 km and administered flunixin meglumine (**FM**) or 0.9% saline (**TRANS**) at loading (d 0) and upon arrival (d 1), or non-transported steers administered 0.9% saline (**CON**). A treatment × day interaction was detected ($P = 0.03$). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ($P = 0.02$), b = FM vs. CON ($P \leq 0.01$), c = TRANS vs. FM ($P = 0.04$).

During feedlot receiving, mean plasma cortisol concentrations tended to be greater in TRANS vs. FM ($P = 0.09$) and CON steers ($P = 0.08$; 41.3, 35.9, and 35.4 ng/mL, respectively; SEM = 2.2), but was similar ($P = 0.87$) between CON and FM steers (treatment effect; $P = 0.09$). These results indicate that flunixin meglumine prevented the increase in circulating cortisol concentrations elicited by long road transport (Cooke et al., 2011; Marques et al., 2012). Serum NEFA concentrations were greater ($P < 0.02$) for TRANS and FM vs. CON on d 1, and greater ($P < 0.04$) for FM vs. TRANS and CON on d 4 (Figure 1; treatment × day interaction, $P = 0.03$). These results corroborate that road transport stimulate fat tissue mobilization and increase circulating NEFA concentrations in cattle (Marques et al., 2012). In addition, flunixin meglumine administration further increased this response, given that serum NEFA concentrations in FM steers were still elevated on d 4 relative to CON and TRANS cohorts. The reason for the increased serum NEFA concentrations in FM steers compared to CON and TRANS cohorts is still unknown, given that the effects of flunixin meglumine on lipid metabolism in beef cattle still need investigation.

A treatment x day interaction was detected for plasma haptoglobin ($P < 0.01$; Figure 2), whereas a tendency ($P = 0.10$; Figure 3) for the same interaction was detected for plasma ceruloplasmin. Plasma haptoglobin concentrations were greater ($P < 0.01$) for TRANS vs. CON and FM on d 1 and 4, and greater ($P < 0.05$) for FM vs. CON on d 1 and 4. Plasma ceruloplasmin concentrations were greater ($P < 0.03$) for TRANS vs. CON on d 1, 4, and 7, greater ($P < 0.05$) for TRANS vs. FM on d 4 and 7, and greater ($P < 0.04$) for FM vs. CON on d 1 and 4. Previous research from our group also documented an acute-phase protein reaction in beef cattle upon a similar 24-h road transport (Araujo et al., 2010) that impaired feedlot receiving performance (Marques et al., 2012). Accordingly, circulating concentrations of acute-phase proteins in transported feeder cattle have been negatively associated with feedlot receiving performance (Araujo et al., 2010), and such outcome can be attributed to altered basal metabolism, increased tissue catabolism, and reduced feed efficiency during an acute-phase response (Johnson, 1997).

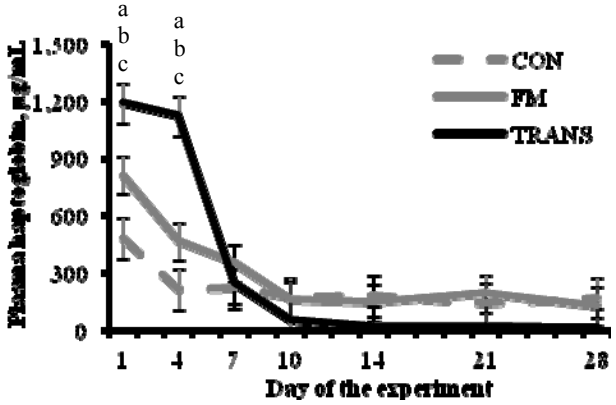

Figure 2. Plasma haptoglobin concentrations in steers transported for 1,280 km and administered flunixin meglumine (**FM**) or 0.9% saline (**TRANS**) at loading (d 0) and upon arrival (d 1), or non-transported steers administered 0.9% saline (**CON**). A treatment × day interaction was detected ($P < 0.01$). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ($P < 0.01$), b = FM vs. CON ($P \leq 0.05$), c = TRANS vs. FM ($P < 0.01$).

Supporting our hypothesis, flunixin meglumine administration alleviated the acute-phase protein response elicited by transport. This outcome can be attributed to the

decreased plasma cortisol concentrations during feedlot receiving in FM steers, given that cortisol stimulates the bovine acute-phase protein reaction (Cooke and Bohnert, 2011). In addition, flunixin meglumine inhibits cyclooxygenase, an enzyme that regulates synthesis of inflammatory eicosanoids associated with the acute-phase response such as PGE_2 (Odensvik, 1995). However, in the present experiment, FM and TRANS steers had a similar decrease in feedlot receiving performance compared to that of CON cohorts, indicating that flunixin meglumine administration reduced the acute-phase protein response but did not alleviate the performance losses caused by road transport. Still, the acute-phase reaction during feedlot receiving may negatively impact performance (Cooke et al., 2009) and increases the incidence of respiratory diseases in overtly healthy cattle. Therefore, the development of management strategies that prevent or alleviate the acute-phase response during feedlot receiving, including flunixin meglumine administration, is warranted for optimal performance, health, and efficiency parameters in feedlot systems. Perhaps a greater dosage of flunixin meglumine, such as 2.2 mg/kg of BW at loading and upon feedlot arrival, is necessary to further reduce the transport-elicited acute-phase protein response and enhance performance parameters.

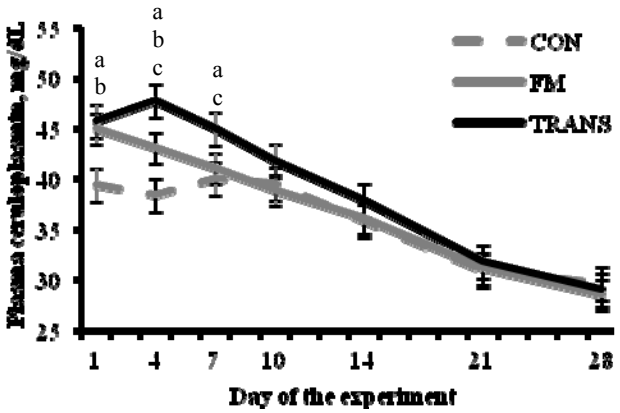

Figure 3. Plasma ceruloplasmin concentrations in steers transported for 1,280 km and administered flunixin meglumine (**FM**) or 0.9% saline (**TRANS**) at loading (d 0) and upon arrival (d 1), or non-transported steers administered 0.9% saline (**CON**). A tendency for treatment × day interaction was detected ($P = 0.10$). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ($P < 0.03$), b = FM vs. CON ($P \leq 0.04$), c = TRANS vs. FM ($P \leq 0.05$).

Implications

Flunixin meglumine administration to feeder steers prior to road transport and at feedlot arrival prevented the increase in circulating cortisol and alleviated the acute-phase protein response elicited by transport, but did not improve feedlot receiving performance. Hence, flunixin meglumine appears to be a viable alternative to reduce neuroendocrine and acute-phase protein responses during feedlot receiving. Therefore, additional research is warranted to further assess the benefits of flunixin meglumine administration, including greater dosages, on health and productive responses of transported cattle.

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MEAN PULMONARY ARTERIAL PRESSURE IN BEEF CALVES IS POSITIVELY ASSOCIATED WITH MIXED VENOUS CO2 TENSION AND OXYGEN EXTRACTION RATIO WHEN CONTROLLING FOR ALVEOLAR OXYGEN TENSION

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ABSTRACT: Beef calves may have a high O_2 demand at a time when their lungs are functionally immature. Adequacy of O_2 delivery relative to demand can be estimated from the oxygen extraction ratio (OER), which is the absolute difference in oxyhemoglobin saturation between arterial and mixed venous blood divided by arterial oxyhemoglobin saturation. The effects of mismatch between O_2 demand and O_2 delivery on pulmonary arterial pressure (PAP) are unknown. Chronic alveolar hypoxia is a known risk factor for bovine pulmonary hypertension (BPH). We hypothesized that OER is positively associated with mean PAP when controlling for alveolar O_2 tension. Arterial and mixed venous blood-gas tensions and calculations derived from these indices were evaluated for associations with mean PAP. A total of 122 Angus calves were randomly sampled from 2 herds on 2 occasions at 2,731m (herd A, n = 64) and 2,166m (herd B, n = 58) above sea level. Pulmonary pressures were measured using a fluid-filled catheter. Mixed venous blood and arterial blood were collected from the pulmonary and coccygeal arteries, respectively. Generalized estimating equations were used to account for repeated measures. Herd, sex, age of calf and alveolar O_2 tension were included in the model. Mixed venous CO_2 tension ($P < 0.001$) and OER ($P = 0.02$) were positively associated with mean PAP when controlling for herd ($P = 0.01$), sex ($P = 0.01$), alveolar O_2 tension ($P = 0.002$) and age ($P < 0.001$). A steer with an OER of 0.40 has a mean PAP 14.3 ± 0.9 mmHg higher than an steer with an OER of 0.1 when controlling for herd (B), age (200 days), alveolar O_2 tension (70 mmHg) and mixed venous CO_2 tension (50 mmHg). In conclusion, calves with a high O_2 demand relative to O_2 delivery are at increased risk of BPH. We speculate that continued selection of cattle for metabolically expensive traits, such as rapid weight gain, without concurrent selection for physiologic traits associated with O_2 delivery is predicted to increase the incidence of BPH.

Key words: arterial blood-gas, beef calves, oxygen extraction, pulmonary arterial pressure

INTRODUCTION

Bovine pulmonary hypertension (**BPH**) is historically considered to be a disease of high altitude environments (Rhodes, 2005). The problem was first reported to occur almost 100 years ago (Glover and Newsom, 1915). It was reported to occur at altitudes over 2,134 m (7,000 ft.) (Hecht et al., 1962). Medial hypertrophy of pulmonary arterioles was found to occur in response to chronic low alveolar O_2 tension (Jaenke and Alexander, 1973). Consequently, the reduction in vessel lumen diameter increases vascular resistance and pulmonary arterial pressure (**PAP**). Cardiac failure and death may ensue. This traditional model of BPH pathogenesis does not adequately explain the occurrence of BPH at moderate altitudes. For example, BPH occurrence in yearling Holsteins at 1,600 m (5,249 ft.) (Malherbe et al., 2012). Recent studies prove the pathogenesis to be more complex than previous thought (Frid et al., 2006; Stenmark et al., 2006). It has been speculated that cattle are susceptible to BPH because of their small cardiopulmonary system relative to their basal O_2 requirements (Veit and Farrell, 1978).

The oxygen extraction ratio (**OER**) is the ratio of oxygen consumption to delivery and is typically 0.2 to 0.3 (McLellan and Walsh, 2004). An increase in OER above 0.3 represents an inability of the cardiopulmonary system to deliver sufficient O_2 to meet metabolic demands. We hypothesized that OER is positively associated with mean PAP irrespective of alveolar O_2 tension. Arterial and mixed venous blood-gas tensions and calculations derived from these indices were evaluated for associations with mean PAP.

MATERIALS AND METHODS

The Colorado State University Animal Care and Use Committee approved of the animal handling and sampling procedures prior to sample collection.

Study site

Calves from one herd in southern Wyoming (Herd A) and one herd in south-west Colorado (Herd B) were studied

on 2 occasions (Table 1). Calves were of the Angus breed. Calves tested in herd A included 4 steers and the remainder were bulls. Approximately equal numbers of steers and heifers were tested in herd B.

Table 1. Herd, altitude, date of sampling, number of calves sampled and age

Herd	Altitude, m	Date	n[1]	Mean age ± SD, days
A	2,166	07/31/2012	60	124.0 ± 18.2
		10/01/2012	65	186.7 ± 17.6
B	2,731	06/21/2012	58	85.9 ± 6.7
		10/10/2012	51	197.2 ± 6.6

[1] number of calves sampled

The dams of calves studied were given a pre-breeding and pre-calving vaccination offering protection against *Bovine herpesvirus 1* (infectious bovine rhinotracheitis [IBR]), BVDV, *Bovine respiratory syncytial virus* (BRSV), and *Bovine parainfluenza virus 3* (BPIV-3). Calves were vaccinated against the same respiratory pathogens at 4 to 8 weeks of age and 2 to 4 weeks prior to weaning. Both herds used a modified-live vaccine on both cows and calves (Bovishield Gold 5, Zoetis, Madison, NJ). Calves on both ranches were administered a killed vaccine at 4 to 8 weeks of age offering protection against: *Cl. chauvoei, Cl. septicum, Cl. novyi, Cl. sordellii* and *Cl. perfringens* Type C and D. Vaccines were given according the manufacturers instructions.

In both herds ear notch samples were routinely collected from all calves kept as replacement heifers for *Bovine viral diarrhea virus* (BVDV) enzyme-linked immunosorbent assay testing. No calves persistently infected with BVDV have been detected in these herds to date. Communal grazing with neighboring herds does not occur. Mineral supplements were provided year-round. A hormonal growth promotant (Synovex C, Zoetis, Madison, NJ) containing 100 mg progesterone and 10 mg estradiol benzoate was administered to both heifer and steer calves in herd B when they were approximately 8 weeks old.

Pulmonary arterial pressure measurement and blood sample collection

A full description of the equipment, materials and facilities required for PAP testing is provided by Holt and Callan (2007). In brief, a large bore needle is inserted into the jugular vein. Flexible catheter tubing is then fed through the needle, into the cardiac right atrium, into the right ventricle, and then into the pulmonary artery. A pressure transducer connects the saline-filled catheter to an oscilloscope. The position of the catheter is determined from the pressure waveform on the oscilloscope. The jugular vein, right atrium, right ventricle and pulmonary artery have distinct pressure waveforms.

After the measurement of PAP the catheter was disconnected from the transducer. Saline within the catheter was suctioned out using a 12 ml syringe. Approximately, 2 to 2.5 ml of mixed venous blood from the pulmonary artery was collected in a 3 ml syringe for all of the blood-gas analyses performed. Blood was collected from the coccygeal artery using a 22 gauge, 2.54 cm (1") hypodermic needle. The bovine coccygeal artery is a suitable source for blood-gas analysis (Collie, 1991; Nagy et al., 2002). Arterial blood, unlike venous blood, can fill a heparinized syringe without applying suction. Therefore, minimal, if any, negative pressure was applied to the syringe chamber by drawing on the plunger when obtaining a sample. Syringes were heparinized with approximately 0.25 ml of sodium heparin (1,000 IU/ml). The plunger of each syringe was pulled back to the 3 ml mark coating the inner chamber surface with heparin. Heparin was then expelled so that only the needle hub contained heparin. Collection of blood up to the 2 ml mark resulted in dilution of the blood sample with sodium heparin (1,000 IU/ml) by < 5 %. Blood dilution of < 10 % is sufficient to minimize pre-analytic error (Hutchison et al., 1983). The sample was discarded if during collection the flow of arterial blood was interrupted. Air bubbles within the blood were immediately expelled and the first several drops of blood discarded before analysis.

Blood-gas analysis was performed using a handheld analyser (VetScan i-STAT 1, Abaxis, Union City, CA) within 3 minutes of the blood draw. Results were automatically stored under the animal identification number. A temperature 'correction' algorithm was used to adjust blood-gas tensions according to rectal temperature (CLSI, 2001). Variables evaluated for association with mean PAP included: pH, pO_2, pCO_2, oxyhemoglobin saturation (**$sHbO_2$**) and L-lactate. Alveolar O_2 tension was estimated from the alveolar gas equation (Fenn et al., 1946). The OER was calculated as absolute difference in oxyhemoglobin saturation between arterial and mixed venous blood divided by arterial oxyhemoglobin saturation.

Statistical Procedures

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, TX). Generalized estimating equations were used to account for the repeated measures (Liang and Zeger, 1986; Zeger and Liang, 1986). An exchangeable correlation structure was used. Mean PAP was positively skewed and so was transformed (PAP^-2.15) into a normal distribution. Herd was included as a fixed effect to account for clustering. The covariate age was included in the model to account for functional maturity of the cardio-pulmonary system (Lekeux et al., 1984). Alveolar O_2 tension (**p_AO_2**) was included in the model to account for the vasoconstrictive effect of alveolar hypoxia (Sylvester et al., 2012). Arterial and mixed venous blood-gas variables (pH, pCO_2, pO_2 and $sHbO_2$), OER and L-lactate were tested for association with mean PAP while controlling for herd, age and p_AO_2. All variables with an association ($P \leq 0.25$)

were included in a backwards elimination model. A type I significance level of 0.05 was used for the final model. Two-way interactions between all variables in the final model were also evaluated.

RESULTS

Mixed venous CO_2 tension ($P < 0.001$) and OER ($P = 0.02$) were positively associated with mean PAP when controlling for herd ($P = 0.01$), sex ($P = 0.01$), p_AO_2 ($P = 0.002$) and age ($P < 0.001$) (Figure 1). Correlation between mixed venous CO_2 tension and arterial CO_2 tension was low but significant ($r = 0.26$, $P < 0.001$). A steer with an OER of 0.40 has a mean PAP 14.3 ± 0.9 mmHg higher than an steer with an OER of 0.1 when controlling for herd (B), age (200 days), alveolar O_2 tension (70 mmHg) and mixed venous CO_2 tension (50 mmHg) (Table 2).

Table 2. Predicted mean pulmonary arterial pressures (PAP) for calves with oxygen extraction ratios (OER) of 0.1 and 0.4 when controlling for age (200 days), alveolar O_2 tension (70 mmHg) and mixed venous CO_2 tension (50 mmHg).

		OER 0.10	OER 0.40
Ranch	Sex	Mean PAP ± SE, mmHg	Mean PAP ± SE, mmHg
A	Bull	59.8 ± 39.3	72.1 ± 38.3
	Steer	48.7 ± 40.3	54.4 ± 39.3
B	Heifer	56.4 ± 39.3	66.3 ± 38.4
	Steer	62.0 ± 38.5	76.4 ± 37.5

DISCUSSION

Striking parallels can be drawn between BPH and pulmonary hypertension of broiler chickens. Mean PAP is positively associated with growth rate in beef calves (Shirley et al., 2008) and broiler chickens (Peacock et al., 1989). Pulmonary hypertension was first reported to occur in both calves and broilers at high altitude (Cueva et al., 1974; Glover and Newsom, 1915). It is now reported to occur in herds at moderate altitude (Malherbe et al., 2012) and flocks at sea level (Peacock et al., 1990). Pulmonary hypertension in broilers is the product of a physiological imbalance. Broilers have inadequate pulmonary (Wideman et al., 2007) and/or cardiac capacity (Olkowski et al., 2005) to deliver sufficient O_2 to meet requirements. It has been suggested that the small cardiopulmonary capacity of cattle relative to O_2 requirements is a risk factor for BPH (Veit and Farrell, 1978). Growth of broilers is positively associated with OER and mixed venous CO_2 tension (Olkowski et al., 2005). Here, we provide evidence that the same relationship exists for beef calves.

IMPLICATIONS

We speculate that continued selection of cattle for metabolically expensive traits, such as fast growth, without concurrent selection for physiologic traits associated with O_2 delivery will increase the incidence of BPH. Pulmonary hypertension is estimated to cost the broiler industry $1 billion per year (Currie, 1999). The cost of BPH to the cattle industry is likely to be considerable given that BPH is not a disease exclusive to high altitude and current mitigation strategies, although beneficial, are not solving the problem (Neary, 2013).

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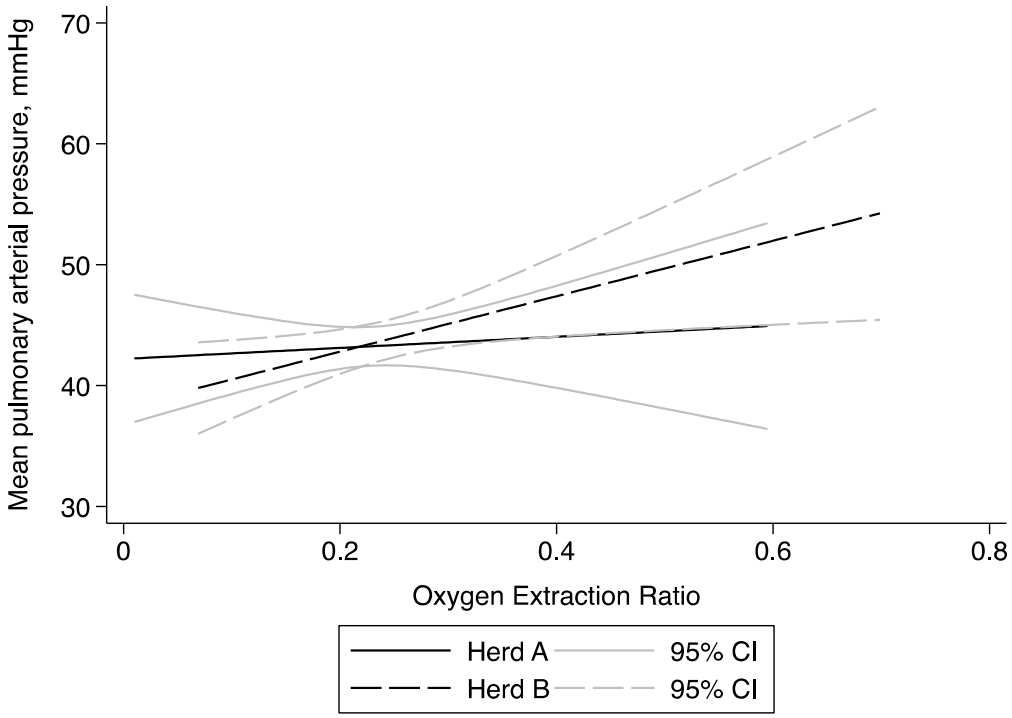

Figure 1. Mean pulmonary arterial pressure (mmHg) by herd and oxygen extraction ratio. Regression lines are provided with 95% confidence intervals (95% CI) of the mean. Calves in herd A and herd B were tested at altitudes of 2,166m and 2,731m, respectively.

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THE EFFECT OF DIET AND FEED EFFICIENCY STATUS ON RUMEN MICROBIAL PROFILES IN SHEEP

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ABSTRACT: The rumen microbial ecosystem plays a large role in fermentation of consumed feeds in ruminant livestock, and therefore may influence the efficiency of feed utilization. The objective of this study was to determine the effects of diet and feed efficiency status on rumen microbial profiles in growing lambs. Growing wethers (initial BW = 51.3 ± 1.2 kg; n = 77) of Rambouillet, Hampshire, and Suffolk breed types were randomly allocated to receive a concentrate- (n = 39) or forage-based (n = 38) pelleted diet. Lambs were acclimated to diets over a 25 d period. Individual feed intake was measured with a GrowSafe System for 49 d and BW was recorded weekly to allow for estimation of residual feed intake (RFI), a measure of feed efficiency. Rumen fluid samples were collected at the end of the feeding trial, and DNA was extracted for sequencing from the rumen fluid of the four most (low RFI) and four least efficient (high RFI) wethers on each diet. Paired-end reads were filtered, quality trimmed and compared with a database of known 16S rRNA genes. Operational taxonomic units (OTU) were defined as sequence clusters with ≥ 97% identity; 349 prokaryotic OTUs were present in at least one animal. The GENMOD procedure of SAS was used to determine the effects of diet, feed efficiency status and their interaction on OTU abundance using a Poisson distribution. The most abundant OTU across diets was *Prevotella ruminicola*, which had greater ($P < 0.001$) abundance in forage-fed lambs compared with concentrate-fed lambs. In total, there were 83 OTUs that differed ($P \leq 0.05$) by diet, and 49 of those were of greater ($P \leq 0.05$) abundance in concentrate-fed wethers. Additionally, 29 OTUs differed ($P \leq 0.05$) according to RFI status (i.e. low versus high RFI), with 17 of those OTUs having greater ($P \leq 0.05$) abundance in high RFI lambs. Finally, there were 33 OTUs affected ($P \leq 0.05$) by the interaction between diet and RFI status; 27 of these OTUs were in greater ($P \leq 0.05$) abundance in low RFI, concentrate-fed lambs. Results from this study indicate that diet influences the rumen microbiome. Furthermore, key rumen microbial species may play an important role in the regulation of feed efficiency, and those species may differ according to diet composition.

Key words: feed efficiency, lambs, microbes, rumen, sequencing

Introduction

Ruminal microbiota regulate the fermentation of feedstuffs and end-products that are utilized by the host. They have a significant effect on host maintenance, growth and performance. In turn, the host provides an ideal anaerobic environment and substrate for microbiota to thrive, generating a mutualistic relationship for both host and microbiota populations. Several factors can affect microbial composition in the rumen including type and composition of feed, age and health of host, environmental temperature and seasonality, and geographic location (Tajima et al., 2001; Romero-Perez et al., 2011; Carberry et al., 2012). It is known that diet is the main determinant of rumen microbial composition; however, there is currently limited understanding of ruminal bacterial communities in livestock, especially as related to feed efficiency.

Methane production by ruminants has become a global concern. Approximately 33% of total worldwide methane emissions are attributed to livestock enteric fermentation and manure (Mamuad et al., 2012). Furthermore, ruminants can lose between 5.5-9.0% of their ingested energy through gas production in the rumen during fermentation of feedstuffs (Zhou et al., 2011). Reducing methane production in ruminants would not only contribute to decreased methane emissions, but also improved feed efficiency and productivity.

Because feed costs for livestock are a substantial portion of production costs, improving feed efficiency becomes more important as feed costs continue to rise. Improvements in feed efficiency can translate to reducing feed usage while maintaining animal performance. Residual feed intake (RFI) is a measurement of feed efficiency that is defined as the difference between the actual and predicted feed intake as it relates to observed ADG (Koch et al., 1963). There is a great deal of research related to RFI in cattle; however, little work has been reported on RFI in sheep. Similarly, work relating RFI with ruminal microbial profiles has been limited to-date. Relationships between RFI and microbial profiles could facilitate selection of breeding stock based on feed efficiency without the need to collect individual feed intake data, an expensive and time-intensive task. The objective of this study was to determine the effects of diet and feed efficiency status on rumen microbial profiles in growing lambs. We hypothesized that microbial profiles would differ between diets as well as feed efficiency status.

Materials and Methods

Animals and Diet. All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. Growing wethers (n = 77; initial BW = 51.3 ± 1.2 kg) of Rambouillet, Hampshire, and Suffolk

breed types were randomly allocated by BW to receive either a concentrate (CONC; n = 39) or forage-based (FOR; n = 38) pelleted diet (Table 1). Lambs were acclimated to diets using a 20% increase in proportion of new feed to old feed every 4-5 d until the diet consisted of 100% new pelleted diet *ad libitum*. Individual feed intake was measured using the GrowSafe System for a 49 d trial period. Two-day average initial and final BW were obtained to calculate ADG. From these data, RFI was calculated as the deviation of true feed intake from expected feed intake. Expected feed intake was determined by regressing ADG and metablic midweight on actual feed intake (Cammack et al., 2005). Residual feed intake calculations were used to rank wethers on efficiency. Rumen fluid samples were collected via oral lavage, allocated in triplicate into 2 mL tubes, snap-frozen on dry-ice, and stored at -80º C until processing.

DNA Extraction from Rumen Fluid. DNA was extracted from the rumen fluid of the most efficient (n = 8; low RFI) and least efficient (n = 8; high RFI) wethers. Zirconia (0.3 g of 0.1 mm) and silicon (0.1 g of 0.5 mm) beads and 1 mL lysis buffer were added to thawed rumen fluid samples and tubes were homogenized using a Mini-Beadbeater-8 at maximum speed for 3 min, incubated at 70° C for 15 min with gentle mixing every 5 min, and centrifuged at 4° C for 5 min. Supernatant was transferred to a new 2 mL flat cap tubes and fresh lysis buffer was added to the pelleted beads. The homogenization, incubation and centrifugation were repeated and the supernatants were pooled. Precipitation of nucleic acids, removal of RNA and proteins, and purification were completed using the protocol of the QIAamp DNA Stool Mini Kit (Qiagen, Santa Clarita, CA).

Microbial Sequencing. Extracted DNA was sent to the University of Missouri (Columbia) DNA Core facility for sequencing using 16 libraries of an Illumina HighSeq platform, with 4 libraries per lane. The resulting 100 base-pair, paired-end reads were filtered, quality-trimmed, and compared with a database of 27K known 16S rRNA genes using the Bowtie reference-based assembly tool. Operational taxonomic units (OTU) were defined as sequence clusters with ≥ 97% identity.

Statistical Analysis. The MIXED procedure of SAS was used to determine the effect of diet on feed intake, ADG, and G:F using data from all wethers (n = 77). A generalized linear model was fitted using the GENMOD procedure of SAS (SAS Inst. Inc., Cary, NC) to determine the effects of diet, feed efficiency status, and their interaction on OTU abundance using a Poisson distribution. Raw *P*-values from the Poisson regression were corrected for multiple tests using the false-discovery rate correction of Benjamini and Hochberg (1995). Post hoc analyses comparing treatment means were conducted using the LSMEANS procedure of SAS.

Results and Discussion

Animal Performance. Average daily feed intake of the FOR-fed wethers (3.14 ± 0.08 kg) was greater ($P \leq 0.001$) than intake of CONC-fed wethers (2.18 ± 0.06 kg). Correspondingly, ADG was also greater ($P \leq 0.001$) in FOR-fed (0.27 ± 0.01 kg/d) compared with CONC-fed wethers (0.20 ± 0.01 kg/d). The G:F ratio was not affected by diet type ($P = 0.23$). Residual feed intake, the measure of feed efficiency, ranged from -0.47 to 0.69 for wethers fed the CONC diet, and from -0.70 to 0.80 for wethers fed the FOR diet.

Diet. There were 349 prokaryotic OTUs present in at least one animal. In total, there were 83 OTUs that differed ($P \leq 0.05$) by diet, and 49 of those were of greater ($P \leq 0.05$) abundance in CONC-fed wethers. The most abundant OTU across diets was *Prevotella ruminicola* (Table 2), which had greater ($P < 0.001$) abundance in FOR-fed lambs compared with CONC-fed lambs. *Prevotella ruminicola* encompasses one of the most numerous groups of rumen bacteria. These bacteria utilize a wide variety of carbohydrates and are therefore represented in ruminants fed a variety of different diets (Chapman and Hall, 1997). *Prevotella ruminicola* is a nonstructural carbohydrate fermenter that can ferment and utilize cellulose, hemicellulose, pectin, starch, sugars and proteins (Van Soest, 1994). This may explain the great overall abundance of this bacterial species in the rumen, but does not explain the lower abundance in CONC-fed wethers.

Among CONC-fed lambs, *Dialister succinatiphilus* and *Acidaminococcus fermentans* were the most abundant OTUs ($P \leq 0.05$), followed by several *Prevotella* species. In total, 21 out of 31 *Prevotella* species that differed ($P < 0.05$) by diet were of greater abundance ($P < 0.05$) in CONC-fed lambs. There were 5 *Clostridium* species that differed ($P \leq 0.05$) by diet, and 3 were greater ($P \leq 0.05$) for CONC than FOR-fed lambs. There were three methanogen-producing species affected by diet; one unknown *Methanobrevibacter* species was expectedly in greater ($P \leq 0.001$) abundance in CONC-fed wethers, but the other two (*Methanobrevibacter smithii* and *Methanospheara stadtmanae)* were greater ($P \leq 0.001$) in FOR-fed lambs.

RFI Status. Additionally, 29 OTUs differed ($P \leq 0.05$) according to RFI status (low RFI versus high RFI), with 17 of those OTUs having greater ($P \leq 0.05$) abundance in high RFI lambs (Table 2). Out of the 16 *Prevotella* species that differed ($P \leq 0.05$) with RFI status, 11 were greater ($P \leq 0.05$) in high RFI lambs. There were 3 *Ruminococcus* species that differed ($P \leq 0.05$) by diet, and 2 were in greater ($P \leq 0.01$) abundance in high RFI than low RFI lambs. One methanogen producing species, *Methanobrevibacter smithii*, differed by RFI status and was in greater ($P \leq 0.01$) abundance in low RFI wethers.

Interaction of Diet and RFI Status. Finally, there were 33 OTUs affected ($P \leq 0.05$) by the interaction between diet and RFI status; 27 of these OTUs were in greater ($P \leq 0.05$) abundance in low RFI, CONC-fed lambs. Interestingly, when OTU abundance was greater in low RFI, CONC-fed lambs, the abundance was typically the lowest among the low RFI, FOR-fed lambs. Similarly, when high RFI, FOR-fed lambs had the greatest OTU abundance, in turn the high RFI, CONC-fed lambs had the lowest abundance. This suggests that rumen microbial populations important to feed efficiency are also largely dependent upon diet. Finally, when low RFI, CONC-fed lambs did not have the greatest OTU abundance among all interaction combinations, the high RFI, FOR-fed animals

typically possessed the greatest OTU abundance. For example, of 18 *Prevotella* species that differed ($P \leq 0.05$) with the diet and RFI interaction, the low RFI, CONC-fed lambs had greatest OTU abundance for 14 of those species. Interestingly, *Mitsuokella jalaludinii* was greater ($P < 0.001$) in low RFI, CONC-fed lambs. *Mitsuokella jalaludinii* has been demonstrated as an efficient methane-reducing agent in the rumen by competing with methanogens for hydrogen, necessary for growth by both (Mamued et al., 2012).

Discussion. Results from this study indicate that diet influences the rumen microbiome. Furthermore, key rumen microbial species may play an important role in the regulation of feed efficiency, and those species may differ according to diet composition. In addition, broad groups of microbes, such as *Prevotella sp.*, *Clostridium sp.*, or *Methanobrevibacter sp.*, may not give many indications of these influences, but rather specific individual species do, such as *Methanobrevibacter smithii*. Moreover, methane-reducing species, including *Mitsuokella jalaludinii* may not only decrease gas production in livestock, but also improve ruminal fermentation, and in turn, improve feed efficiency. This may suggest that the favorable relationship between better feed efficiency and lower methane production may be due to an increased ability to reduce methane in the rumen as opposed to lower abundance of actual methanogenic species. Further research is necessary to determine the types of species that may play this role, and whether they can be influenced to further decrease methane production, especially in livestock on high concentrate diets. A better understanding of the influence of diet type, RFI status and their interaction on the rumen microbiome is needed to determine if animal feed efficiency is translatable across different production settings (e.g., grazing versus feedlot).

Implications

Prediction of feed efficiency status without the need to measure individual feed intake is a necessary step in realizing the potential of this economically important trait as a tool for genetic selection. Use of rumen microbial populations as a means of assessing feed efficiency may ultimately provide producers with an easier (i.e. one-time rumen sampling) and more affordable means of identifying feed efficient breeding stock, especially as technologies, such as DNA sequencing, continue to become less expensive. Finally, it will be important to recognize that the microbial species that play a key role in the regulation of feed efficiency likely differ with diet composition.

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Table 1. Composition of pelleted diets.

Item	FOR[1]	CON[2]
Ingredient, % DM		
Alfalfa pellets	67.70	--
Corn	--	50.20
Wheat middlings	27.50	31.00
Corn gluten	--	10.00
Cane molasses	2.50	2.50
Salt	1.34	1.76
Calcium carbonate	0.60	2.30
Dried distillers grains with solubles	--	1.00
Calcium sulfate	--	0.75
Potassium chloride	--	0.19
Trace minerals and vitamins[3]	0.34	0.36
Analyzed nutrient composition		
DM, % as fed	92.30	91.60
CP, % DM	16.20	12.10
NDF, % DM	36.30	17.60
ADF, % DM	25.10	6.60
ME, Mcal/kg[4]	2.31	2.98
Ca, % DM[4]	1.20	1.30
P, % DM[4]	0.37	0.47

[1]FOR = foraged-based pelleted diet.
[2]CONC = concentrate-based pelleted diet.
[3]Includes Selenium 1600, Sheep TM ORG-Zn, Flavor APF-168, Vit E 20000 IU/#, and CHS/PN VT-FDLT.
[4]Calculated from NRC (2007) values.

Table 2. Least-squares means of microbiota abundance in lambs identified as feed efficient or feed inefficient on a concentrate- or forage-based pelleted diet

	Diet*Residual Feed Intake (RFI) Status				*P*-value
Microbiota	CON[1]*High[2]	CON*Low[3]	FOR[4]*High	FOR*Low	Diet*RFI
Acidaminococcus fermentans	149.75^{b}	452.75^{a}	12.75^{c}	14.50^{c}	< 0.001
Bifidobacterium dentium	0.50^{c}	189.50^{a}	2.50^{b}	0.50^{c}	< 0.001
Bifidobacterium longum	0.50^{b}	40.5^{a}	1.50^{b}	1.00^{b}	< 0.001
Butyrivibrio hungatei	1.75^{b}	6.00^{a}	9.75^{a}	9.75^{a}	0.047
Butyrivibrio species	1.25^{b}	5.75^{a}	3.00^{ab}	1.25^{b}	0.005
Clostridium clostridioforme	8.25^{b}	34.25^{a}	2.50^{c}	1.00^{c}	0.001
Eubacterium eligens	32.25^{b}	147.00^{a}	3.25^{c}	1.75^{c}	< 0.001
Mitsuokella jalaludinii	15.50^{b}	122.25^{a}	6.50^{c}	3.75^{c}	< 0.001
Prevotella albensis	37.25^{b}	63.25^{a}	29.00^{c}	20.00^{d}	< 0.001
Prevotella amnii	12.75^{bc}	19.50^{a}	15.25^{ab}	9.50^{c}	0.006
Prevotella bivia	6.75^{a}	8.75^{a}	8.00^{a}	2.00^{b}	0.003
Prevotella brevis	94.00^{b}	190.25^{a}	204.50^{a}	204.25^{a}	< 0.001
Prevotella genomospecies[5]	143.25^{a}	100.50^{b}	46.25^{c}	89.25^{b}	< 0.001
Prevotella genomospecies	5.00^{d}	33.00^{c}	77.25^{a}	47.25^{b}	< 0.001
Prevotella genomospecies	1.00^{d}	4.00^{c}	17.25^{a}	9.25^{b}	0.004
Prevotella micans	1.75^{d}	12.25^{c}	33.75^{a}	20.00^{b}	< 0.001
Prevotella oris	48.50^{b}	79.75^{a}	16.75^{c}	16.00^{c}	0.026
Prevotella oulorum	10.50^{a}	12.25^{a}	11.00^{a}	4.25^{b}	0.009
Prevotella paludivivens	6.25^{a}	8.75^{a}	8.25^{a}	2.75^{b}	0.006
Prevotella pleuritidis	20.25^{a}	17.50^{a}	0.75^{c}	4.00^{b}	0.023
Prevotella ruminicola	32.00^{d}	127.00^{c}	957.50^{a}	645.25^{b}	< 0.001
Prevotella salivae	2.50^{b}	6.00^{a}	6.00^{a}	1.75^{b}	0.001
Prevotella species	125.50^{a}	135.00^{a}	12.50^{b}	2.25^{c}	< 0.001
Prevotella species	3.25^{b}	6.25^{ab}	9.00^{a}	5.75^{ab}	0.047
Prevotella stercorea	23.00^{b}	40.50^{a}	15.00^{c}	7.50^{d}	< 0.001
Prevotella timonensis	5.75^{b}	11.75^{a}	11.00^{a}	4.00^{b}	< 0.001
Ruminococcus albus	5.50^{d}	11.50^{c}	66.75^{a}	44.50^{b}	< 0.001
Ruminococcus callidus	12.75^{a}	17.75^{a}	5.75^{b}	1.75^{c}	0.006
Ruminococcus torques	5.75^{b}	25.00^{a}	3.00^{bc}	2.00^{c}	0.001
Schwartzia succinivorans	0.50^{c}	7.75^{a}	3.50^{b}	2.00^{bc}	< 0.001
Selenomonas bovis	15.50^{b}	176.00^{a}	2.75^{c}	0.75^{d}	< 0.001
Selenomonas ruminantium	1.50^{b}	9.00^{a}	2.25^{b}	1.00^{b}	0.003
Selenomonas ruminantium	0.75^{b}	3.75^{a}	5.50^{a}	3.00^{a}	0.011

[1]CON = concentrate-based pelleted diet.
[2]High = high RFI status (low efficiency).
[3]Low = low RFI status (high efficiency).
[4]FOR = forage-based pelleted diet.
[5]Microbiota with the same name are different unknown species within a genus.
Tests of significance generated using the GENMOD procedure of SAS modeled with a Poisson distribution. Treatment means were generated using the MIXED procedure are valid, but because the data were not normally distributed, standard errors are not valid and thus not included.

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MATERNAL NUTRIENT RESTRICTION FOLLOWED BY REALIMENTATION DURING EARLY TO MID-GESTATION ON MAMMARY GLAND DEVELOPMENT IN BEEF COWS

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ABSTRACT: Objectives were to examine effects of early to mid-gestation maternal nutrient restriction followed by realimentation on mammary gland development. On d 30 of pregnancy, multiparous, non-lactating cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) were assigned to dietary treatments in a completely randomized design: control (C; 100% NRC; n = 18) and restricted (R; 60% NRC; n = 28). On d 85, cows were slaughtered (C, n = 6; R, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12), or were realimented to control (RC; n = 11). On d 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n = 6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254 all remaining cows were slaughtered. The diet consisted of grass hay to meet 100% or 60% NE recommendations for maintenance and fetal growth and to meet or exceed MP recommendations. Prior to slaughter, jugular blood samples were collected. Monthly blood samples were collected only for cows slaughtered on d 254 and analyzed for prolactin (PRL). At slaughter, mammary glands were immediately removed and weighed. Glands were analyzed for fat, cellular proliferation, and qPCR was used to determine mRNA expression of vascular endothelial growth factor (VEGF) and its receptors (fms-related tyrosine kinase 1 (FLT1) and kinase insert domain receptor (KDR)). Mammary gland weight was not affected ($P \geq 0.15$) by treatment. There was no treatment effect for PRL at slaughter and no treatment × day interaction ($P \geq 0.27$) for PRL across gestation. Fat (%) did not differ ($P \geq 0.35$) at d 85 and 140; however, at d 254 RRC and RCC cows had less ($P = 0.02$) fat vs. CCC. There was no effect ($P \geq 0.45$) of maternal dietary treatment on mammary alveolar cellular proliferation. There was no treatment effect ($P \geq 0.27$) on mRNA expression of VEGF, FLT1, and KDR. Nutrient restriction during early to mid-gestation does not appear to impact mammary gland weight; however, composition may be altered. Further information is needed to determine how nutritional interventions could improve lactation in beef cattle.

Keywords: Beef cattle, mammary gland, nutrient restriction

INTRODUCTION

Beef cows are commonly managed in grazing systems where forage quality varies according to regional conditions. Forage quality or availability is often poor, affecting nutritional and physiological status of the animal (Funston et al., 2010). During this period of reduced nutrient availability, the dam will undergo a series of metabolic and physiologic adaptations to protect her body stores from depletion as the increase in nutrient demands by the conceptus occurs (Rosso and Streeter, 1979).

Maternal nutrition during pregnancy not only plays an important role for fetal and placental growth and development, but mammary development as well. In order to continue to nourish the offspring after birth, the mammary gland needs to be properly developed. Mammary gland milk production depends on several factors; one of them is the amount of secretory cells (i.e., alveoli) in the gland that secretes milk (Anderson et al., 1985). In addition, maternal nutrition also affects milk composition and production (Miranda et al., 1983). Our laboratory (Swanson et al., 2008; Vonnahme et al., 2011) previously reported that nutritional plane during gestation decreased mammary gland size and proliferation, and altered mammary gland vascularity in sheep. Meyer et al. (2011) reported decreased colostrum and milk production in nutrient restricted ewes; moreover, this decrease in milk production continued after ewes were realimented to control diets during lactation (Meyer et al., 2011). We hypothesize that longer nutrient restriction would negatively impact maternal mammary gland development compared with controls. Our objectives were to determine the effects of realimentation after maternal nutrient restriction during early to mid-gestation on mammary gland development.

MATERIALS AND METHODS

All procedures involving animals were approved by the North Dakota State University (NDSU) Animal Care and Use Committee (#A10001).

Animals, Diets, and Breeding

A total of 54 non-lactating, multiparous crossbred beef cows of similar genetic background were synchronized using a Select Synch plus progesterone insert (CIDR; Pfizer Animal Health, New York, NY) and fixed-time AI (TAI) protocol. At the NDSU Beef Research and Teaching Unit (Fargo, ND), cows were assigned to 1 of 6 breeding groups with breeding dates ranging from July 13th to October 24th 2011. Cows received GnRH (100 µg as 2 mL of Factrel i.m.; Fort Dodge Animal Health, Fort Dodge, IA) and a CIDR on d 0. On d 7 CIDR devices were removed, and cows were given an injection of PGF2α (25 mg as 5 mL of Lutalyse i.m., Pharmacia & Upjohn Co., Kalamazoo, MI). Estrotect Heat Detectors (Rockway Inc., Spring Valley, WI) were used to monitor estrous behavior for a minimum of 72 h. Artificial insemination was performed utilizing the AM/PM rule 12 h after the first detected estrus. Cows not

detected in estrus after 72 h received a second GnRH injection and TAI was performed. Inseminated cows were transported to the Animal Nutrition and Physiology Center (ANPC; Fargo, ND) within 3 d post-insemination. From arrival at ANPC until confirmed pregnant, cows were grouped in pens (n = 4 to 5/pen) and trained to use the Calan gate feeding system. At this time, all cows were fed chopped grass hay [8.02% CP, 69.2% NDF, 41.5% ADF, and 57.9% TDN (DM basis)] and a mineral and vitamin supplement to meet NE recommendations for maintenance and fetal growth and to meet or exceed recommendations for MP, minerals, and vitamins (NRC, 2000) until pregnancy was confirmed. Hay NE_m concentration was predicted using equations described by Weiss (1993) and NRC (2000).

On d 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). Non-pregnant cows restarted the same breeding protocol; cows were only allowed to be AI twice during the experiment. On d 30 of pregnancy, cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) were randomly assigned to dietary treatments: control (C; 100% NRC; n = 18) and nutrient restriction (R; 60% NRC; n= 28). On d 85 cows were slaughtered (**C**, n = 6 and **R**, n = 6), remained on control (**CC**; n = 12) and restricted (**RR**; n = 12) treatments, or were realimented to control (**RC**; n = 11). On d 140 cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (**CCC**, n = 6; **RCC**, n = 5), or were realimented to control (RRC, n = 6). On d 254 all remaining cows were slaughtered (CCC, n = 6; RCC, n = 5; RRC, n = 6).

The control diet consisted of grass hay (Table 1) to meet 100% NE recommendations for maintenance and fetal growth (NRC, 2000) and to meet or exceed MP recommendations. Nutrient restricted cows received 60% of the same control hay diet. Cows were individually fed once daily in a Calan gate system at 1000 and had free access to water. The mineral and vitamin supplement (Trouw dairy VTM with optimins; Trouw Nutrition International, Highland, IL) was top-dressed 3 times per week at a rate of 0.18% of hay DMI to meet or exceed mineral and vitamin requirements relative to dietary NE intake (NRC 2000). Cows were weighed weekly at approximately 0800 throughout the experiment and dietary intake was adjusted relative to BW.

Blood Sample Collection and Analysis

Prior to slaughter, jugular blood samples were collected. In addition, monthly blood samples were collected only for cows slaughtered on d 254 by jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) and allowed to clot at room temperature for 15 min before being placed on ice. Samples were centrifuged at 4°C for 15 min at 1,500 × g and serum was stored at −20°C in plastic vials until assayed. Serum concentrations of PRL were quantified by double-antibody RIA with a detection limit of 2 ng/mL (Spoon and Hallford, 1989).

Slaughter Procedure and Mammary Gland Collection and Processing

On d 85, 140, and 254, a randomly selected subset of cows from each treatment were slaughtered at the NDSU Meat Laboratory. Immediately upon exsanguination, the mammary gland was removed, weighed, and processed. From one-half of the gland, approximately 5 g of glandular tissue from the mammary gland was snap frozen in super-cooled isopentane (submerged in liquid nitrogen) and stored at −80°C until analysis for mRNA expression and fat content (Neville et al., 2010).

A perfusion fixation procedure with Carnoy's fixative (60% ethanol, 30% acetic acid, 10% chloroform) was performed on the remaining half of the mammary gland by cannulating the cranial mammary artery with a polyethylene (PE-60; o.d. = 1.22 mm; i.d. = 0.77 mm; Intramedic, Becton Dickinson and Company, Sparks, MD) beveled catheter that was secured to surrounding tissue. The perfusion fixation procedure consisted of initial perfusion with PBS (in order to remove red blood cells for histology), then with Evan's blue dye (to define the vasculature, and sampling area), and then, finally, with Carnoy's fixative.

Mammary tissue was then cut into approximately 1-cm cubes and was further immersion fixed in Carnoy's fixative for an additional 6 h. Thereafter, mammary gland tissues were dehydrated in a graded series of ethanol and Histo-Clear (National Diagnostics, Atlanta, GA), and embedded in paraffin wax.

Mammary Gland Fat Content by Ether Extract

Approximately 0.5 g of frozen mammary gland sample and its duplicate where freeze-dried and analyzed for lipid contencntration using the ether extract method (920.39; AOAC, 1997). The analysis CV was 4.2%. The percent fat was calculated as followes: ((oil weight) / (sample weight x DM)) × 100.

Measurement of Mammary Gland Cellular Proliferation

Carnoy's fixed tissues were transferred to a graded series of distilled water and then stored in ethanol solution until further processed and embedded in paraffin, sectioned at 4 μm and mounted onto glass slides, rinsed several times in PBS containing Triton-X100 (0.3%, vol/vol) and then treated for 20 min with blocking buffer (PBS containing normal horse serum [2%, vol/vol]), followed by incubation overnight at 4 °C with a specific primary antibody against Ki67 (1:500; mouse monoclonal; Vector Laboratories, Burlingame, CA, USA), an endogenous marker of proliferating cells. The following day, the slides were washed with tris-buffered saline with added Tween 20 (TBST) and incubated with 1:100 goat anti-mouse IgG-fluorescein isothiocyanate secondary antibody for 1 h in complete darkness. A final wash with TBST was performed and coverslips were applied using mounting medium containing 4', 6-diamidino-2-phenylindole (DAPI) in order to visualize cell nuclei. Photomicrographs of 5 random areas per slide were taken with a Zeiss Iamger.M2 epifluorescence microscope using a 10x objective and

AxioCam HRm camera (Zeiss, Thornwood, NY). Images were then analyzed using the ImagePro Plus software (Media Cybernetics, Bethesda, MD, USA) for labeling index (LI, percentage of cells stained with Ki-67).

RNA Isolation, cDNA Synthesis, and Quantitative PCR (qPCR)

Maternal mammary gland mRNA was analyzed for relative expression of vascular endothelial growth factor (***VEGF***) and its receptors (fms-related tyrosine kinase 1 (***FLT1***) and kinase insert domain receptor (***KDR***)). Mammary tissue (30 mg) was homogenized using a Polytron fitted with a 7 mm generator. Following homogenization, RNA was isolated using the Qiagen All Prep kit according to manufacturer's instructions. RNA was quantified on a Nanodrop 2000c spectrophotometer. For cDNA synthesis, 1 µg of RNA from each sample was used in a reverse transcription reaction using the Qiagen QuantiTect RT Kit. Gene expression was determined using Taqman primer/probe sets designed for each gene and qPCR. Within each sample, expression of every gene was normalized to 18s rRNA. The comparative Ct method was used to analyze data and mRNA expression is displayed relative to the control group within each time point.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS software version 9.2, SAS Inst., Cary, NC). Class statement included cow and treatment. Model statement included treatment. Concentrations of PRL from cows slaughtered at d 254 were examined using repeated measures analysis of the mixed procedure. When a significant treatment effect was detected ($P \leq 0.05$), treatment differences were separated using the PDIFF option of the LSMEANS statement.

RESULTS

Mammary gland weight was not affected ($P \geq 0.15$) by dietary treatment. Average mammary gland weights, regardless of dietary treatment, were 4.52 ± 0.59, 3.31 ± 0.46, and 6.20 ± 0.44 kg at d 85, 140, and 254 of gestation, respectively. Similarly, mammary gland weight did not differ ($P \geq 0.16$) when expressed relative to eviscerated BW. Mammary gland fat (%) did not differ ($P \geq 0.35$) among groups at d 85 (35.1 ± 12.1%) and 140 (31.6 ± 14.8%); however, at d 254 RRC and RCC cows had less ($P = 0.02$) fat vs. CCC (12.65 ± 2.2 and 13.88 ± 2.4 vs. 22.06 ± 2.2%, respectively).

There was no dietary treatment effect ($P \geq 0.77$) for PRL at d 85, 140, and 254 of gestation (62.6 ± 30.8, 139.5 ± 86.3, and 58.8 ± 25.37 ng/mL, respectively). When PRL was measured monthly across gestation on cows slaughtered at d 254, there was no treatment × day interaction ($P = 0.27$). However, there was a day effect ($P < 0.01$) for PRL with greater ($P < 0.05$) concentrations on d 180 compared to d 30 and 240.

There was no effect ($P \geq 0.45$) of maternal dietary treatment on mammary alveolar cellular proliferation at d 85 (average = 0.74 ± 0.15%), 140 (average = 0.82 ± 0.14%), and 254 (average = 0.82 ± 0.12%) of gestation. There was no dietary treatment effect ($P \geq 0.27$) on mRNA expression of *VEGF*, *FLT1*, and *KDR* at d 85, 140, and 254 of gestation.

DISCUSSION

The only mammary gland parameter measured in the current study which was influenced by nutrient restriction was fat content within the gland. Perhaps a depletion of this energy source would negatively impact milk performance. Our laboratory (Swanson et al., 2008; Vonnahme et al., 2011) has previously reported in sheep fed 60% of nutrient recommendations during gestation had decreased mammary gland size and proliferation, and altered mammary gland vascularity compared to control ewes. In the current study, maternal nutrient restriction in beef cows followed by realimentation did not affect maternal mammary gland weight or cellular proliferation. It is important to note that the beef cow mammary gland might be less sensitive to nutrient restriction compared to sheep at the time points that we have investigated. In addition, the restriction periods in Swanson et al. (2008) and ours are different and perhaps will have a different impact on mammary gland responses. Nutrient restriction in cross bred dairy cows from 2 wk before calving to 11 wk postpartum resulted in decreased mammary gland weight, and lower number of mammary cells compared to control diets. However, mammary gland epithelial cell proliferation was not affected by nutrient restriction (Dessauge et al., 2010). Since there is more mammary gland growth after parturition, perhaps we did not investigate glandular growth long enough.

Previously, Swanson et al. (2008) showed that maternal nutrient restriction during late gestation decreased postpartum colostrum, which matches the decreased mammary gland weight of the underfed ewe. In addition, cellular proliferation in the alveoli of mammary glands from nutrient restricted ewes was decreased, while the alveolar area was increased (Swanson et al., 2008). In the current study, bovine mammary cellular proliferation was not altered by nutrient restriction followed by realimentation from early to mid-gestation. Neville et al. (2010) had previously reported that mammary glands from ewes that were restricted to 60% of NRC recommendations had an increase in VEGF mRNA expression. In the present study, we did not alter mRNA expression of VEGF and its receptors due to nutrient restriction during early to mid-gestation.

In sheep, the mammary gland grows exponentially during pregnancy and continues during early lactation until peak lactation and it is controlled by hormones (Anderson et al., 1985). Mammary gland growth is slow during early pregnancy; but as pregnancy advances, the growth is accelerated (Anderson et al., 1985). Prolactin plays an important role in maintenance of mammary gland function (Flint and Knight, 1997) and synergistically with other mammotrophic factors, can control mammary gland development (Brisken et al., 1999). In our study, maternal nutrient restriction followed by realimentation did not affect PRL concentrations prior to slaughter or through gestation.

Maternal nutrient restriction during early to mid-gestation followed by realimentation does not appear to impact mammary gland weight; however, fat content was decreased. Our laboratory is currently analyzing mammary gland samples and serum samples for vascularity and other hormones involved in mammary gland development.

IMPLICATIONS

In order to continue to nourish the offspring after birth, the mammary gland needs to be properly developed. Optimization of growth and development of the calf after birth is important for the profitability of the livestock industry. In our study, nutrient restriction during early gestation appears to alter mammary gland fat content without affecting weight and cellular proliferation. More research is necessary to further understand the effects of nutrient restriction followed by realimentation on mammary gland development and milk composition in beef cows.

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Table 1. Nutrient analysis of grass hay

Ingredient	
DM, %	94.3
Ash, % of DM	11.8
CP, % of DM	8.1
NDF, % of DM	69.2
ADF, % of DM	41.5

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EFFECTS OF MATERNAL NUTRITION AND RUMEN-PROTECTED ARGININE SUPPLEMENTATION ON EWE AND POSTNATAL LAMB PERFORMANCE[1]

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ABSTRACT: Our hypothesis was that arginine supplementation would overcome the negative effects of restricted maternal intake during the last two-thirds of gestation on ewe and lamb performance. To test this hypothesis, multiparous, Rambouillet ewes (n = 32) were allocated to 3 treatments in a completely random design at 54 ± 3.9 d of gestation. Dietary treatments were 100% of requirements (control, CON), 60% of control (restricted, RES), or RES plus a rumen-protected arginine supplement dosed at 180 mg/kg BW once daily (RES-ARG). Ewes were penned individually in a temperature-controlled facility. At parturition, lambs were immediately removed from their dam and reared independently. Ewe BW from d 75 of gestation through parturition was greater ($P \leq 0.05$) in CON compared with RES or RES-ARG. Similarly, ewe BCS from d 82 of gestation through parturition was greater ($P \leq 0.02$) in CON than either RES or RES-ARG. Total ewe colostrum (g) at 3 h after parturition was greater ($P \leq 0.0001$) in CON than RES or RES-ARG. Lamb birth weight was greater ($P = 0.04$) in CON than RES ewes, and tended ($P = 0.10$) to be greater in CON vs. RES-ARG. Lambs born to CON ewes had greater ($P \leq 0.03$) BW than lambs from RES ewes at 7, 14, and 33 d postpartum. On d 19, lambs from CON and RES-ARG ewes both had greater ($P \leq 0.04$) BW than lambs from RES ewes (12.0 and 11.5 vs. 10.3 ± 0.41 kg, respectively). Lambs born to CON and RES-ARG ewes had greater ($P \leq 0.04$) ADG than lambs from RES ewes on d 19 (355.0 and 354.0 vs. 306.4 ± 15.77 g, respectively). Lambs from CON and RES-ARG ewes also had greater ($P \leq 0.02$) girth circumference than lambs from RES ewes on d 19 (55.4 and 54.6 vs. 51.3 ± 0.97 cm, respectively). On d 54, lambs from RES-ARG ewes had greater ($P = 0.003$) curved crown rump length than lambs from RES ewes (99.8 vs. 93.9 ± 1.28 cm, respectively). These results confirm our hypothesis that arginine supplementation during the last two-thirds of gestation can mitigate some negative consequences associated with restricted maternal nutrition in the offspring, but not in the underfed dams themselves.

Key words: arginine, developmental programming, gestation, nutrition, offspring

INTRODUCTION

Fetal intrauterine growth restriction (**IUGR**) has been implicated as the cause of many deleterious postnatal offspring performance defects or traits, including lower birth weights and poor neonatal growth and body composition (Wu et al., 2006; Caton and Hess, 2010; Reynolds and Caton, 2012). One of the major causes of fetal IUGR is compromised maternal nutrition, which may occur in extensive grazing systems. In the Western U.S., grazing ewes often receive less than 50% of NRC recommendations, resulting in loss of body weight during pregnancy and reduced lactation performance (Wu et al., 2006). A potential supplement to offset IUGR is arginine, a semi-essential amino acid. Arginine contributes to nitric oxide and polyamine production, both of which play key roles in placental growth and function (Martin et al., 2001; Kwon et al., 2003; Wu et al., 2009). Nitric oxide and polyamines play unique roles in regulating fetal development throughout gestation, and contribute to nutrient delivery to, and use by, the developing fetus despite maternal under nutrition. Improved fetal growth has been demonstrated in ovine models of IUGR in response to intravenous arginine administration (Wu et al., 2009). Use of current rumen protection technologies allows for oral administration of specific amino acids with subsequent delivery to the small intestine, which is a practical approach for strategic supplement delivery to ruminants. In this study, we tested the hypothesis that arginine supplementation would mitigate the negative effects of compromised maternal nutrition during the last two thirds of gestation on both ewe and lamb performance. We expected lambs from nutrient restricted, arginine supplemented dams to present as normal and therefore to be similar to lambs from control-fed ewes.

MATERIALS AND METHODS

Animals

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. Multiparous Rambouillet-cross ewes (n = 32; 67.7 ± 6.2 kg initial BW) were confirmed pregnant via ultrasound on 41 ± 6.0 d after mating. Ewes were housed individually in a climate controlled facility with free access to water. Ewes were fed a pelleted diet daily at 0800.

[1] Appreciation is expressed to Dr. Chris Schauer, NDSU Hettinger Research and Extension Center, Drs. Reid Redden and Pawel Borowicz, NDSU Department of Animal Sciences, and Fernando Valdez, Kemin Industries, for their assistance with this project.

Weekly ewe BW measurements allowed monitoring of ewe BW change to determine if dietary adjustments were needed. Body condition scores were assessed every two weeks by two or three independent observers.

Experimental Design and Treatments

This experiment was a completely random design. Ewes were randomly assigned to one of three treatments at 54 ± 3.9 d of gestation: 100% of dietary requirements (control, **CON**; based on NRC, 1985, 2007), 60% of control (restricted, **RES**), or RES with the addition of a rumen protected arginine supplement (**RES-ARG**). Supplement provided to the RES-ARG ewes contained 180 mg arginine/kg BW (based on initial BW). Arginine was mixed with 50 g of fine ground corn and fed once daily at 0800 before offering the pelleted diet. Both CON and RES ewes were also provided 50 g of fine ground corn daily, without the added rumen protected arginine. Pelleted diets (Table 1) were fed once daily to ewes on an individual basis, with amounts specific to ewe BW and targeted nutrient supply. Pelleted diets were consumed within 2 h of feeding. Treatments continued until parturition. Two CON and one RES ewe died (2 unknown causes and 1 pneumonia) before parturition. Their data were included in the analyses up to removal from the study.

Table 1. Ingredient and nutrient composition of pelleted diet fed to ewes

Item	%
Ingredient	
Alfalfa meal, dehydrated	34.0
Beet pulp, dehydrated	27.0
Wheat middlings	25.0
Ground corn	8.4
Soybean meal	5.0
Trace mineral premix[1]	0.6
Nutrient composition	
DM	91.9
CP	15.5
NDF	35.8
ADF	20.9

[1]Premix: 18 to 21% Ca, 9% P, 10 to 11% NaCl, 49.3 ppm Se, 700,000 IU/kg Vitamin A, 200,000 IU/kg Vitamin D, 400 IU/kg Vitamin E.

Parturition and Lamb Management

A closely monitored, 24 h lambing protocol was implemented during the expected dates of parturition. At parturition, lambs were not permitted to suckle from ewes, and were removed from dams immediately and reared independently. There were four sets of twins (2 CON, 1 RES, and 1 RES-ARG). At 3 h post parturition, ewes were administered a 1 mL (20 USP units) intramuscular injection of Oxytocin (Vet Tek, Blue Springs, MO) and milked out to determine colostrum weight.

Following removal from the ewe, lambs were towel dried and weighed. Lambs received an intramuscular injection of vitamin A, D, and E (0.5 mL/lamb; 100,000 IU of A, 10,000 IU of D_3, 300 IU of E/mL; Stuart Products, Bedford, TX), and 1 mL of *Clostridium perfringens* types C and D and tetanus vaccine (Essential 3+T, Colorado Serum, Denver, CO) subcutaneously. Finally, the umbilical cord was clipped and dipped in 7% iodine tincture.

Lambs received artificial colostrum (Lifeline Rescue Colostrum, APC, Ankeny, IA), administered at 19.1 mL/kg of lamb birth weight at 0 and 2 h post birth, and 25.5 mL/kg of lamb birth weight at 4, 8, 12, 16, and 20 h post birth to achieve 10.64g IgG/kg lamb birth weight, as previously described (Meyer et al., 2010; Neville et al., 2010).

Lambs were group housed in a climate controlled facility with free access to water. At 24 h post birth, lambs received milk replacer (Super Lamb Milk Replacer, Merrick's Inc., Middleton, WI; DM basis: 24% CP, 30% fat, 0.10% crude fiber, 0.5 to 1.0% Ca, 0.65% P, 0.3 ppm Se, 66,000 IU/kg vitamin A, 22,000 IU/kg vitamin D, and 330 IU/kg vitamin E) ad libitum via bottle until a strong suckling response was observed. Lambs then transitioned to a teat bucket system (Meyer et al., 2010; Neville et al., 2010). In addition to milk replacer, a mixture of long stem mid-bloom alfalfa hay and creep feed (DM basis: 20% CP, 6% fat, 8% crude fiber, 1.4 to 1.9% Ca, 0.4% P, 0.5% to 1.5% NaCl, 0.3 ppm Se, 11,000 IU/kg vitamin A, 6,000 IU/kg vitamin D, and 100 IU/kg vitamin E) were available ad libitum. At 7 d, all tails were docked and male lambs were castrated by banding. At 40 ± 3 d, lambs received an additional 2-mL injection of vitamin A, D, and E as previously described. Lamb BW was measured at birth, 24 h, and 3, 7, and 14, 19, 33, 40, 47, and 54 ± 3 d. Curved crown rump length, measured as the distance from the crown of the head to the rump along the backbone, and girth, measured as the circumference around the rib cage just behind the forelegs, were determined at birth, and at 19 and 54 d. Two lambs died during the experiment of unrelated causes, one at 7 d (RES-ARG), and another at 45 d (RES). Their data were included in the analyses up to removal from the study.

Statistical Analysis

Data were analyzed as a completely random design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NY) with ewe or lamb serving as the experimental unit. Fetal number was included in the model statement and retained if ($P \leq 0.10$). After protection with an overall F-test for treatment ($P \leq 0.10$) means were separated using the LSMEANS procedure of SAS and P-values ≤ 0.05 were considered different.

RESULTS AND DISCUSSION

Ewe Performance

Restricted (RES and RES-ARG) ewes weighed less ($P \leq 0.05$) than CON ewes from d 75 of pregnancy until parturition (Table 2). Similarly, RES and RES-ARG ewes had lower ($P \leq 0.02$) BCS than CON ewes from d 82 throughout parturition, and by d 68 CON ewes had greater ($P \leq 0.03$) BCS than RES-ARG ewes (Table 3). These

results are similar to those reported by Meyer et al., (2010). Changes in ewe BW and BCS in CON and RES ewes indicate our experimental model was performing as predicted and appropriate for testing our hypothesis regarding supplementation of rumen-protected arginine. In this study, the arginine treatment had no rescue effect ($P \geq 0.82$) on ewe BW or BCS.

Colostrum produced at 3 h postpartum by CON ewes was greater ($P \leq 0.0001$) compared with RES and RES-ARG ewes (753.7 vs. 298.6 and 105.4 ± 88.31 g, respectively). Differences observed between CON and RES were expected and reported previously (Wu et al., 2006; Swanson et al., 2008; Meyer et al., 2011). Results also indicated that rumen- protected arginine supplementation did not rescue colostrum yield in restricted ewes. Effects of the arginine supplement used in this study on longer-term lactation responses were not determined.

Lamb Birth Weight and Performance

Lambs from CON ewes had greater ($P = 0.04$) BW at birth than lambs from RES ewes, with lambs from RES-ARG fed ewes being intermediate and similar to both CON and RES (Table 4). This same response was observed for lamb BW on d 7, 14, and 33. On d 3, lambs from CON ewes weighed more ($P \leq 0.02$) than lambs from RES and RES-ARG ewes. On d 19, lambs from CON and RES-ARG ewes weighed more ($P \leq 0.04$) than lambs from RES ewes. Keeping with our hypothesis, these data indicate that arginine may play a role in recovering postnatal BW in lambs from nutritionally compromised dams.

Lamb ADG followed a similar pattern as BW, with lambs from CON and RES-ARG ewes having greater ($P \leq 0.04$) ADG than lambs from RES ewes on d 19 (Table 5).

Girth measurements at birth and d 54 were greater ($P = 0.03$) in lambs from CON compared with RES ewes, with RES-ARG being intermediate and similar to both CON and RES (Table 6). However, on d 19 lambs from CON and RES-ARG ewes had greater ($P \leq 0.02$) girth measurements than lambs from RES ewes. Lambs from RES-ARG ewes had greater ($P = 0.003$) curved crown rump measurements than lambs from RES ewes on d 54, with lambs from CON ewes being intermediate and similar to both RES and RES-ARG. These data support the potential role arginine may play in enhancing offspring growth from underfed dams.

IMPLICATIONS

Ewe performance outcomes were inconsistent with our hypothesis and were not responsive to supplemental rumen-protected arginine during gestation. However, in keeping with our hypothesis, lamb BW, ADG and body size measurements were responsive to maternal rumen-protected arginine supplementation, but not at all times measured. Additional research is needed to further define the effects of supplementation of rumen-protected arginine during gestation on offspring heath and performance outcomes.

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Table 2. Influence of nutrient restriction and arginine supplementation on ewe BW (kg) throughout gestation

	Treatment[1]				
d	CON	RES	RES-ARG	SEM	*P* - value
54	63.8	63.7	64.2	1.95	0.98
61	64.1	60.3	60.9	1.95	0.32
68	62.9	57.8	57.5	1.94	0.09
75	62.3[b]	56.8[a]	56.7[a]	2.00	0.07
82	64.1[b]	57.9[a]	57.3[a]	1.99	0.03
89	65.3[b]	58.1[a]	57.6[a]	2.09	0.02
96	65.5[b]	57.9[a]	57.9[a]	2.20	0.02
103	65.7[b]	57.7[a]	57.4[a]	2.13	0.01
110	66.4[b]	57.7[a]	57.7[a]	2.06	0.005
117	67.0[b]	56.9[a]	56.5[a]	2.16	0.002
124	67.6[b]	56.3[a]	56.7[a]	2.17	0.001
131	67.9[b]	56.0[a]	56.3[a]	2.13	0.001
138	69.6[b]	56.6[a]	56.9[a]	2.12	0.001
145	69.6[b]	56.3[a]	56.1[a]	2.23	0.001
152	67.0[b]	56.5[a]	56.6[a]	3.30	0.06

[1]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 10).
[a, b]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 3. Influence of nutrient restriction and arginine supplementation on ewe BCS throughout gestation[1]

	Treatment[2]				
d	CON	RES	RES-ARG	SEM	*P* - value
54	2.90	2.91	2.88	0.075	0.94
68	2.94[b]	2.78[ab]	2.71[a]	0.073	0.08
82	2.99[b]	2.65[a]	2.66[a]	0.094	0.02
96	2.90[b]	2.47[a]	2.42[a]	0.093	0.001
110	2.93[b]	2.40[a]	2.34[a]	0.137	0.006
124	2.98[b]	2.26[a]	2.26[a]	0.108	<0.0001
138	2.90[b]	2.01[a]	2.05[a]	0.133	<0.0001
152	2.75[b]	1.65[a]	1.79[a]	0.199	0.001

[1]BCS structured by scale of 1 = thin to 5 = over conditioned
[2]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 10).
[a, b]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 4. Influence of maternal nutrient restriction and arginine supplementation on offspring BW (g) over time

	Maternal Treatment[1]				
d	CON	RES	RES-ARG	SEM	*P* - value
0	5,228[b]	4,450[a]	4,603[ab]	257	0.09
3	6,045[b]	4,693[a]	4,990[a]	298	0.01
7	7,112[b]	6,101[a]	6,321[ab]	313	0.07
14	9,882[b]	8,811[a]	9,459[ab]	309	0.05
19	11,973[b]	10,272[a]	11,500[b]	405	0.01
33	17,360[b]	15,278[a]	16,202[ab]	574	0.04
40	19,971	18,072	19,488	706	0.14
47	21,765	20,606	22,028	797	0.41
54	23,830	21,870	23,656	890	0.24

[1]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 11).
[a, b]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 5. Influence of maternal nutrient restriction and arginine supplementation on offspring ADG (g) over time

	Maternal Treatment[1]				
d	CON	RES	RES-ARG	SEM	*P* - value
3	272.4	81.0	129.1	73.0	0.17
7	269.1	235.9	245.4	19.5	0.47
14	332.4	311.5	334.7	13.1	0.37
19	355.0[b]	306.4[a]	354.0[b]	15.8	0.05
33	367.6	328.1	346.3	13.3	0.11
40	368.6	340.6	367.9	14.5	0.28
47	351.9	342.3	367.1	15.0	0.51
54	344.5	321.4	349.7	15.0	0.37

[1]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 11).
[a, b]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 6. Influence of maternal nutrient restriction and arginine supplementation on offspring girth (cm) and curved crown rump (cm) length over time

	Maternal Treatment[1]				
Item	CON	RES	RES-ARG	SEM	*P* - value
Girth					
0d	42.2[b]	38.6[a]	39.4[ab]	1.14	0.08
19d	55.4[b]	51.3[a]	54.6[b]	0.98	0.01
54d	70.8[b]	67.0[a]	69.7[ab]	1.24	0.10
CCR					
0d	54.9	52.6	55.1	1.49	0.43
19d	73.7	69.4	72.9	1.86	0.21
54d	96.3[ab]	93.9[a]	99.8[b]	1.28	0.01

[1]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 11).
[a, b]Means within a row with different superscripts differ ($P \leq 0.05$).

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EFFECTS OF HUMAN CHORIONIC GONADOTROPIN ADMINISTRATION IN EWES POST MATING ON SERUM PROGESTERONE CONCENTRATION AND NUMBER OF CORPORA LUTEA AND CONCEPTI

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ABSTRACT: The objective was to determine if administration of human chorionic gonadotropin (**hCG**) on d 4 after mating would increase serum progesterone (**P4**) concentrations, number of corpora lutea (**CL**), and concepti in ewes. Nineteen mixed-aged western whiteface ewes (mean BW = 70.5 ± 1.5 kg) received an intravaginal pessary impregnated with 20 mg flurogestone acetate for 14 d to synchronize estrus. Ewes were mated with fertile rams at the second estrus after pessary removal and were randomly assigned to 1 of 2 treatments. Ewes received 600 IU (4.8 mL) of hCG i.m. on d 4 (n = 9) or saline (4.8 mL) for control ewes (n = 10) post mating. Within each treatment, ewes were randomly assigned to 1 of 2 groups where half the ewes were euthanized 13 d post mating and the remaining ewes, that were confirmed pregnant, were euthanized 25 d post mating. Jugular blood samples were collected from all ewes starting on d 0 (d 0 = d of P4 levels at 1.0 ng/mL or less) through their designated euthanasia day post breeding (d 13 or 25). On d 13 and 25 post mating, ovaries were collected, and CL counted. On d 25 concepti were also collected and counted. Serum P4 concentrations were similar ($P > 0.05$) between treatments through d 3. Ewes receiving hCG on d 4 had greater ($P < 0.05$) serum P4 concentrations from d 4 to 13. Serum P4 values from d 14 to d 25 in pregnant ewes in the second euthanasia group were also examined (treatment by day interaction, $P > 0.05$). The hCG-treated ewes continued to have greater serum P4 concentrations than controls (10.9 vs. 6.0 ± 0.6 ng/mL, respectively). Ewes receiving hCG had greater number of CL ($P < 0.05$) compared to control ewes. All control ewes had ≤ 3 CL, whereas, 87.5% of ewes that received hCG had ≥ 3 CL. In the control group, only 50% had multiple concepti, where as all hCG-treated ewes ($P = 0.108$) had multiple concepti (d 25 euthanasia group). Therefore, administration of hCG on d 4 post mating increased the number of CL present, elevated serum P4 concentrations, and tended to increase number of concepti retrieved on d 25.

Key words: concepti number, human chorionic gonadotropin, progesterone

Introduction

Embryonic mortality in most mammals is approximately 30%, and of this total loss, 70 to 80% occurs between d 8 and 16 after insemination (Bolet et al., 1985). Several reasons contribute to embryonic loss; however, the prominent theory is insufficient functionality of the CL to produce sufficient P4 levels to support conceptus survival and prime the uterus to become a responsive environment for the developing conceptus (Kittok et al., 1983). Insufficient luteal function (poor P4 synthesis and secretion) is believed to be an important contributor to reproductive failure in mammals. A CL producing suboptimal levels of P4 is most likely an indicator of the dam's inability to maintain and carry the pregnancy (Kittok et al., 1983).

Maternal recognition of pregnancy is essential for maintenance of pregnancy, and in ruminants the primary signal is secretion of interferon tau (IFN-τ) from the trophectoderm between d 10 and 21 of pregnancy. It exerts a paracrine effect on the uterine endometrium to abolish luteolytic mechanism (Spencer et al., 1996). Progesterone acts on the uterus to create a quiescent and conducive environment for the embryo to be successful in development and implantation (Spencer et al., 1996). Treatment with hCG, not only increases the concentrations of P4 ($P < 0.10$), but also the total CL weight 12 and 36 h after administration (Nephew et al., 1994). Our hypothesis is that supplementation of hCG on d 4 after mating increases serum P4 concentrations, number of CL present, and number of concepti. The objective was to determine if administration of human chorionic gonadotropin (**hCG**) on d 4 after mating would increase serum progesterone (**P4**) concentrations, number of corpora lutea (**CL**), and concepti in ewes.

Materials and Methods

Animals and Treatment

All procedures involving animals were approved by the New Mexico State University Animal Care and Use Committee (IACUC #2012-018). Nineteen mixed-aged western whiteface ewes (BW = 70.5 ± 1.5 kg) received an intravaginal pessary impregnated with 20 mg flurogestone acetate (Searle, Skokie, IL, 60076) for 14 d to synchronize estrus. Three vasectomized rams, fitted with marking harnesses, were placed with ewes, for detection of estrus. Ewes were mated with fertile rams at the second estrus after pessary removal and onset of estrus, determined as the day a ewe's P4 was at or below 1 ng/mL (d 0). Ewes were

randomly assigned to 1 of 2 treatments. Ewes received 600 IU (4.8 mL) of hCG (ProSpec-Tany Techno Gene Ltd., Ness Ziona, Israel, Cat #: hor-250) i.m. on d 4 (n = 9) or saline (4.8 mL) for control ewes (n = 10) post mating. Within each treatment, ewes were randomly assigned to 1 of 2 groups where half the ewes were euthanized 13 d post mating and the remaining ewes, that were confirmed pregnant, were euthanized between 22 and 25 d post mating.

Blood Collection and Progesterone Assay

Daily blood samples were collected from all ewes starting on the date of being marked by the ram until euthanasia via jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO). Tubes were held at room temperature for at least 30 min prior to being centrifuged at 4°C for 15 min at 1,500 x g. Serum was harvested into plastic vials and subsequently stored at -20 °C until assayed.

Serum P4 concentrations were determined using RIA procedures described by Schneider and Hallford (1996; Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) and performed by the New Mexico State University Endocrinology Laboratory. Inter- and intra-assay CV were 4.7 and 6.7%, respectively.

Euthanasia

Ewes were held off of feed for 12 h and water 3 h before the euthanasia. Ewes were anesthetized with 20 mg/kg BW of sodium pentobarbital (Vortech Pharmacy, Dearborn, MI, 48126) via i.v. administration. The surgical area was clipped and reproductive tract was removed using a mid-ventral laparotomy prior to euthanasia by exsanguination. One group of ewes was euthanized on d 13, and CL were counted and weighed. In addition uteri were flushed and numbers of concepti were recorded. The second group of ewes was euthanized between 22 and 25 d post mating, and CL were counted and weighed, and concepti numbers were recorded.

Corpora Lutea Measurement and Pregnancy Determination

The first euthanasia group pregnancy status was not determined, and was therefore determined at the time of sacrifice. Each uterine horn was flushed with approximately 20 mL of 10 mM PBS supplemented with 0.25% Fraction V BSA (pH 7.1, obtained via pH meter). All recovered conceptuses were placed into 2.0 ml Eppendorf tubes and briefly centrifuged and any liquid was removed and embryos were weighed. The second euthanasia group had P4 assayed between d 15 and 18 to determine pregnancy status. Elevated serum P4 concentrations, signifying the maintenance of a CL, were the defining characteristic of pregnancy in these ewes. Once this was determined, only pregnant ewes were sacrificed.

Statistical Analysis

Experimental design was a completely randomized design. Corpora lutea number and conceptus number per ewe were analyzed by Chi-Square using the frequency procedure of SAS (SAS Inst. Inc., Cary, NC). Progesterone concentrations were analyzed using the mixed procedure of SAS with repeated function. Treatment and ewe were in the whole plot and day and day by treatment interaction were in the subplot.

Results

A day by treatment interaction ($P < 0.05$) was observed for serum P4 concentrations for all ewes (Table 1) until d 13. Serum P4 concentrations were similar ($P > 0.05$) among treatments through d 3. Ewes receiving hCG on d 4 had greater ($P < 0.05$) serum P4 concentrations than control ewes from d 4 to 13.

For serum P4 concentration between d 14 and day of euthanasia (d 22 to 25; second euthanasia group), no treatment by day interaction was detected ($P > 0.20$). Therefore, treatment effects were examined across sampling days. During this period hCG-treated ewes continued to have elevated ($P = 0.001$) serum P4 values (10.9 ± 0.6 ng/mL) compared to controls (6.0 ± 0.6 ng/mL).

Ewes receiving hCG had more CL ($P = 0.006$) compared to control ewes (Table 2). All control ewes had ≤ 3 CL, whereas, 87.5% of ewes that received hCG had ≥ 3 CL (Table 2). In the control group, only 50% had multiple concepti, where as all hCG-treated ewes ($P = 0.108$) had multiple concepti (d 25 euthanasia group, percentages not shown; Table 3). All ewes administered hCG had 2 concepti retrieved, while in the control ewes 3 had 1 conceptus, 2 controls had 2 concepti, and 1 control had 3 concepti ($P = 0.108$). No difference was observed between mean weight of concepti for control and hCG-treated ewes (339.7 and 473.6 ± 74.7 mg, respectively, $P = 0.20$).

Discussion

Data from this study support our hypothesis that supplementation with hCG on d 4 after mating increases serum P4 concentrations and number of CL present. Administration of hCG on d 4 increased serum P4 concentrations, similar to other research conducted in our lab (Yates et al., 2009, Lankford et al., 2010, and Richardson et al., 2011). Also, in the present study ewes receiving hCG had an increased number of CL similar to data by Cam and Kuran (2004), in which ewes treated with 150 IU hCG on 12 d post mating had 2.2 times more CL and greater CL weight ($P < 0.05$) than control ewes. Khan et al. (2007) observed that hCG treatment, on d 12 of pregnancy increased luteal weight ($P < 0.05$) compared to control ewes, on d 25 of pregnancy. Farin et al. (1988) found that administration of hCG or LH increased number of accessory CL present on d 10 as well as CL from the preceding estrus. Because hCG-treated ewes had more CL than controls, in the present study it is assumed that additional CL resulted from hCG causing ovulations of developing follicles or luteinization of these follicles (Khan et al., 2009). Therefore, the increased serum P4

concentrations can be explained by increased number of CL.

Our study found that the number of concepti was not greater in hCG-treated ewes; however, all of these ewes had multiple concepti where as 3 of 6 control ewes had a single conceptus. Cam and Kuran (2004) reported an increase in concepti number when ewes were administered 150 IU hCG 12 d post mating. These researchers observed that ewes administered hCG and GnRH had more twins ($P < 0.05$) than control ewes. Thirty six hours after pessary removal, 150 IU hCG, administered to ewe lambs increased ($P < 0.05$) the number of lambs born (Khan et al., 2003). Nephew et al. (1994) found that administration of 100 IU of hCG on d 11.5 post mating resulted in longer concepti (3.5 ± 1.6 cm vs. 0.8 ± 0.5 cm; $P < 0.05$), and higher concentrations of protein and IFNτ was found in uterine flushing. Ewes treated with hCG also had greater pregnancy rates compared to control ewes, 94% and 83%, respectively (Nephew et al., 1994). Khan et al. (2007) observed enhanced luteal weight, amniotic sac width and length, crown-rump length, embryo weight, and number of placentomes in ewes administered hCG compared to control ewes.

Implications

Data reported herein supports our hypothesis that administration of hCG to ewes on d 4 after mating increased serum P4 concentrations and number of CL, while maintaining a pregnancy of multiple fetuses. Therefore, administration of hCG on d 4 post-mating has the potential to increase lamb crop percentages.

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Table 1. Serum progesterone (P4) concentrations (ng/mL) for ewes treated with human chorionic gonadotropin (600 IU; i.m.) 4 d post mating compared to control ewes.[1]

	Treatment[2]			
Day[3]	Control	hCG	SE[4]	*P* – Values[5]
0	0.8	0.7	0.13	0.612
1	0.5	0.4	0.07	0.415
2	0.6	0.7	0.07	0.287
3	1.0	1.2	0.10	0.245
4	1.4	1.9	0.15	0.048
5	2.1	3.3	0.18	< 0.001
6	2.9	4.9	0.31	< 0.001
7	3.7	7.0	0.35	< 0.001
8	4.5	9.4	0.69	< 0.001
9	4.9	10.4	0.69	< 0.001
10	5.2	11.3	0.65	< 0.001
11	6.1	13.5	0.74	< 0.001
12	5.8	13.2	0.84	< 0.001
13	6.6	13.0	1.05	< 0.001

[1]Ewes were synchronized for estrus using an intravaginal progestogen containing insert (flurogestone acetate soaked sponge) for 14 d. Fertile rams were placed with ewes on the second estrus after sponge removal.

[2]Ewes in the control group received 4.8 mL saline i.m.; ewes in the hCG group received 600 IU of hCG i.m. in 4.8 mL of saline 4 d post mating. A treatment x day interaction ($P < 0.05$) was noted; therefore, treatment means were compared within day.

[3]Day 0 corresponds to the day of mating or day that P4 concentrations were below 1 ng/mL.

[4]Most conservative standard error (n = 9).

[5]Probability values

Table 2. Number of corpora lutea (CL) in ewes treated with human chorionic gonadotropin (hCG 600 IU; i.m.) 4 d post mating compared to control ewes.[1]

	CL Number[3]			
Treatment[2]	1	2	3	4
Control	4	5	1	0
hCG	0	1	1	6

[1]Ewes were synchronized for estrus using an intravaginal progestogen containing insert (flurogestone acetate soaked sponge) for 14 d. Fertile rams were placed with ewes on the second estrus after sponge removal.

[2]Ewes in the control group received 4.8 mL saline i.m.; ewes in the hCG group received 600 IU of hCG i.m. in 4.8 mL of saline 4 d post mating. Chi-Square ($P = 0.006$).

[3]Values represent number of ewes.

Table 3. Number of concepti in ewes treated with human chorionic gonadotropin (hCG 600 IU; i.m.) 4 d post mating compared to control ewes in euthanasia group 2.[1]

	Concepti Number[3]		
Treatment[2]	1	2	3
Control	3	2	1
hCG	0	4	0

[1]Ewes were synchronized for estrus using an intravaginal progestogen containing insert (flurogestone acetate soaked sponge) for 14 d. Fertile rams were placed with ewes on the second estrus after sponge removal. Ewes were euthanized between d 22 to 25 post mating.

[2]Ewes in the control group received 4.8 mL saline i.m.; ewes in the hCG group received 600 IU of hCG i.m. in 4.8 mL of saline 4 d post mating. Chi-Square (P = 0.108).

[3]Values represent number of ewes.

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EFFECT OF KETAMINE-STUN AND MELOXICAM ON BEHAVIOR AND PERFORMANCE ASSOCIATED WITH BAND CASTRATION IN CULL BEEF BULLS

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ABSTRACT: Our objectives were to evaluate the effects of ketamine-stun (**KET**) and oral meloxicam (**MEL**) at the time of band castration on performance and behavioral response of Angus bulls immediately post-weaning. Angus bulls (n = 119, BW 291.3 ± 29.1 kg) were blocked by BW in a complete randomized design via a 2 × 2 factorial arrangement with one additional treatment group remaining intact. Bulls to be castrated were randomly assigned to one of 4 treatments: 1) meloxicam with no ketamine-stun (**MEL**), 2) meloxicam and ketamine-stun (**MEL+KET**), 3) ketamine-stun with no meloxicam (**KET**), or 4) no meloxicam or ketamine-stun (**CON**). Meloxicam was administered on d 0, 7, and 14 (3 mg/kg) via oral bolus. Ketamine stun consisted of butorphanol (0.0125 mg/kg), xylazine (0.025 mg/kg), ketamine (0.054 mg/kg) and was administered 10 min before band application via a single subcutaneous injection. Animals not receiving KET or MEL received a subcutaneous injection of saline or an empty bolus, respectively. Bulls that remained intact were subjected to sham manipulation of the scrotum associated with castration, but without band application. Range of vertical head motion (**DIST**) during castration or sham was used as a behavioral pain indicator during castration. Animals were observed at 3 min intervals for 15 min immediately post castration or sham to evaluate behavior response. Performance measurements analyzed over the 28-d period included ADG, G:F, and DMI. Castrates tended ($P = 0.06$) to have greater DIST than bulls. There was an interaction ($P = 0.001$) among main effects for DIST. The CON group had greater ($P < 0.01$) DIST than all other treatments. Mean percent lying down immediately post-castration was greater ($P < 0.001$) for castrates than bulls and greater ($P < 0.001$) for KET vs. no KET. Bulls exhibited greater G:F ($P = 0.02$) and ADG ($P < 0.001$) than castrates. There was no effect of KET or MEL on G:F ($P \geq 0.12$) or ADG ($P \geq 0.13$). In conclusion, castration resulted in more head motion, particularly in animals not receiving analgesia or anesthesia. Further, post-castration behavior was altered due to castration and the use of KET. Data suggest that neither KET nor MEL alter feed performance among castrated bulls.

Key Words: Animal welfare, Beef bulls, Castration, Ketamine, Meloxicam

Introduction

Castration of bull calves is a common livestock management practice in the United States with approximately 7 million calves castrated annually (USDA, 2009). However there has been increased interest by the public about the ethical treatment of livestock. A Gallup poll in May 2003 revealed that 75% of the general public favors laws regulating treatment of farm animals (Rollin, 2004). The author also reported that consequently, public perception of pain associated with castration has been increasingly negative. There has been amplified pressure to develop practices to mitigate pain and suffering.

One of the confounding issues associated with pain mitigation is there are currently no analgesic drugs approved for pain relief in livestock by the United States Food and Drug Administration (**FDA**; Smith and Modric, 2013). According to the FDA, "validated methods of pain assessment must be used in order for a drug to be indicated for pain relief in target species" (FDA-CVM, 2009). However, validated methods of pain assessment are extremely limited in the scientific literature. Pain assessment in prey animals, such as cattle, is complex given the instinctual reaction to conceal pain (Underwood, 2002). The hypothesis of the current study was that treatment with sub-anesthetic ketamine-stun (**KET**) and nonsteroidal anti-inflammatory drug (**NSAID**) meloxicam (**MEL**) would decrease the negative impact of castration on behavior and performance response of cull beef bulls. The objective was to evaluate the effects of KET and oral MEL at the time of band castration on performance and behavioral response of Angus bulls immediately post-weaning.

Materials and Methods

Animals This project was approved by the Institutional Animal Care and Use Committee at Colorado State University. One hundred and nineteen recently-weaned Angus bulls (BW 291.3 ± 29.1 kg) from a seedstock operation were used. Thirty five bulls remained intact and were randomly assigned to 1 of 4 pens (**BULL**). The remaining 84 bulls to be castrated were blocked by BW (heavy and light) and randomly assigned in a 2 × 2 factorial design to 1 of 4 treatments: 1) MEL, 2) MEL+KET, 3) KET, or 4) no analgesia/anesthesia (**CON**). Each treatment consisted of 4 pens with 5 or 6 animals per pen.

Data Collection. All animals were fed a total mixed ration. Feed was delivered once daily at 0800. Refusals were recorded daily by a trained bunk reader, to supply ad libitum feed. Orts were collected weekly and weighed in order to calculate DMI. Orts were then composited and a representative sample was dried for 48 h in a forced air oven at 60ºC to determine percent DM. Nutrient analysis of orts was conducted by a commercial

laboratory (SDK Laboratories, Hutchinson, KS). Performance measurements analyzed over the 28-d period included ADG, G:F, and DMI. Body weight was collected on d -7, 0, 1, 7, 14, 21 and 28.

Procedures. Bulls to be castrated received a band on d 0. Bulls that remained intact were subjected to sham manipulation of the scrotum associated with castration, but without band application. Band castrations were completed by securing a latex band around of the neck of the scrotum to cause necrosis of the scrotum and testis using a Calicrate Bander (No Bull Enterprises, St. Francis, KS).

Castrates receiving MEL were administered 3.0 mg/kg on d 1, 7, and 14, respectively, by rounding to the nearest tablet (Meloxicam 15 mg, Zydus Pharmaceuticals, Pennington, NJ). Tablets were then encapsulated in a porcine gelatin bolus (Torpac Inc., Fairfield, NJ) and administered via a stainless steel balling gun. All cattle not receiving MEL were given a placebo of an empty gelatin capsule.

Castrates receiving KET were administered a single subcutaneous injection consisting of butorphanol (0.0125 mg/kg), xylazine (0.025 mg/kg), and ketamine (0.054 mg/kg) approximately 10 min prior to band application. Animals not receiving KET received a subcutaneous injection of saline.

Behavioral Measurements. Video documentation at the time of castration or sham was collected for each bull. Video data were analyzed using video analysis software (Dartfish INC., Alpharetta, GA). This was a modified version of collection as noted by Schartzkopf-Genswein et al. (1998), in which they examined the effect of hot-iron, freeze, and sham branding on head movement and velocity. The current experiment examined the maximum vertical range of head movement (**DIST**) during the procedure as noted by differences in highest and lowest points of the nose in the frame of the video. In addition, animals were observed by 2 trained observers blinded to the study at 3 min intervals for 15 min immediately post band application or sham to evaluate behavior response (**OBS**). At each 3 min time point observers classified each animal's current behavior as either standing or laying.

Statistical Analyses. Pen was the experimental unit for all data analyses. And, the alpha level was set at $P = 0.05$. Average daily gain, G:F, DMI, and DIST were analyzed via SAS using two-way analysis of variance with factors being ketamine and meloxicam (PROC MIXED, SAS Institute, Cary, NC). Consistent with our objectives, data were analyzed in a $2 \times 2 + 1$ factorial design to determine the effect of castration and the main effects of MEL and KET using single degree of freedom contrasts. If an interaction among the main effects (MEL and KET) was present ($P < 0.10$), the 4 castrate treatment means were compared.

Results and Discussion

Video analysis at the time of castration or sham indicated that castrates tended ($P = 0.06$, Table 1) to have greater DIST than bulls. Among castrates, there was an interaction ($P = 0.001$) between main effects for DIST. As a result, means for the 4 castrate treatments were compared individually, and the CON group had greater ($P < 0.01$) DIST than all other treatments. Head movement associated with castration revealed that animals being castrated tended to have a greater resistance behavior than sham castration.

Although the effect of KET on head movement was expected due to the sub-anesthetic effects of ketamine-stun, it is inexplicable why MEL administered directly before band application affected head movement. Although all animals received an oral bolus immediately prior to band or sham application, it is possible that the extra weight of the meloxicam tablets influenced the head movement of the animals in the MEL treatment groups. A previous study noted differences in DIST across method of branding (hot-iron, freeze, and sham) with hot-iron branding eliciting the greatest DIST response (Schwartzkopf-Genswein et al., 1998). Though the current study examined castration, we can assume based on the previous study that DIST provides indication of pain response.

Mean percent of animals lying down during the 15-min period immediately post-castration was greater ($P < 0.001$) for castrates than bulls. There was no interaction ($P = 0.17$) between main effects, however there was an interaction ($P = 0.0003$) between time and the main effects. Mean percent of animals lying was greater ($P < 0.001$; Figure 1) for KET vs. no KET across all time points starting at 3 min ($P = 0.05$; Table 2). There was no effect ($P = 0.58$) of MEL on lying behavior at any time point. None of the sham bulls were observed lying down during the observation period.

Post-castration observations indicated KET increased the percent of cattle that lied down immediately after castration. A study in 2008 using accelerometers to evaluate standing and lying behavior of cattle both before and after surgical castration found that calves spent significantly more time standing post-surgical castration (White et al., 2008). Although the method of castration was different, data reported by the authors suggested that KET altered standing behavior. The results from the current study were also observed in a study using accelerometers in conjunction with KET during surgical castration. Treatment with KET and sodium salicylate in that study increased the lying behavior immediately following castration (Pauly et al., 2012). Increased lying behavior could also be attributed to the potential sedative effect of the sub-anesthetic combination of xylazine, ketamine, and butorphanol, although it is intended to provide sedation without recumbency in cattle (Coetzee, 2011). Additionally previous work indicated that more calves treated with ketamine and xylazine displayed unchanged attitude following castration compared to non-treated controls (Coetzee et al., 2010).

Dry matter intake did not differ ($P > 0.34$, Table 3) between bulls and castrates. There was no interaction ($P = 0.80$) between main effects for DMI. There were no effects of KET ($P = 0.14$) or MEL ($P = 0.93$) on DMI. Bulls exhibited greater ADG ($P < 0.001$; Table 3) and G:F ($P = 0.02$) than castrates. There was no interaction ($P = 0.60$) between main effects and no effect of KET or MEL on G:F ($P \geq 0.12$) or ADG ($P \geq 0.13$).

Average daily gain and G:F of castrates, regardless of treatment, was less than for bulls. However, DMI among

castrates did not differ from the bulls. This suggests that stress of castration negatively impacted feed efficiency and gain for the 28-d span after band application. Use of analgesia and sub-anesthetic did not mitigate these negative effects in the current study. This is similar to results of a previous study that investigated oral MEL in association with surgical castration (Coetzee et al., 2011). The authors suggested that MEL impacted some aspects of morbidity, but no behavioral or feedlot performance parameters were different when compared to other castrates.

Implications

Based on behavior and feedlot performance response variables, limited conclusions can be drawn about the effects of meloxicam and ketamine-stun on reducing negative impacts resulting from castration. Further investigation into the use of pain mitigation with castration on performance and behavior is necessary in order to address increasing demand by the public for humane production of beef. Increased knowledge on pain mitigation in livestock will be necessary to validate the usage of pain mitigation drugs in the future.

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Table 1: Least square means (± SEM) for vertical head movement (DIST) in response to castration and main effects of subcutaneous ket-stun and oral meloxicam after band castration or sham in weaned beef bulls

	Treatment[1]						Contrast, *P* =			
Item	BULL	CON	KET	MEL	MEL+KET	SEM	CAST[2]	INT[3]	KET	MEL
DIST[6] (m)	0.31[a]	0.60[b]	0.39[a]	0.28[a]	0.38[a]	0.062	0.06	0.001	0.12	0.01

[1]BULL = sham procedure on d 0, CON = band on d 0, KET = band on d 0 with subcutaneous ket-stun, MEL = band on d 0 with oral meloxicam, MEL+KET = band on d 0 with subcutaneous ket-stun and oral meloxicam.
[2]CAST = Contrast between pooled CON, KET, MEL, MEL+KET means and BULL mean.
[3]INT = Interaction of main effects (KET and MEL).
[4]KET = Contrast between KET and non-KET treatments.
[5]MEL = Contrast between MEL and non-MEL treatments.
[6]Head movement at the time of castration was determined using video analyzing software in which the greatest difference in nose position longitudinally was measure during band castration or sham castration.
[ab] Means without common superscripts differ ($P < 0.05$).

Table 2: Least square means (± SEM) for percent of pen lying down at 3 min intervals for a 15-min period post castration and main effects of subcutaneous ket-stun and oral meloxicam after band castration or sham in weaned beef bulls

	Treatment [1]						Contrast, *P* =			
Time	BULL	CON	KET	MEL	MEL+KET	SEM	CAST[2]	INT[3]	KET[4]	MEL[5]
0 min	0.00	0.00	0.00	0.00	0.00	0.13	-	-	-	-
3 min	0.00	0.00	0.30	0.00	0.28	0.14	0.35	0.95	0.05	0.95
6 min	0.00	0.00	0.50	0.10	0.43	0.11	0.05	0.45	0.002	0.88
9 min	0.00	0.04	0.60	0.10	0.48	0.12	0.04	0.49	0.003	0.82
12 min	0.01	0.05	0.76	0.11	0.63	0.10	0.02	0.30	<0.001	0.68
15 min	0.00	0.13	0.80	0.15	0.72	0.09	0.004	0.58	<0.001	0.58

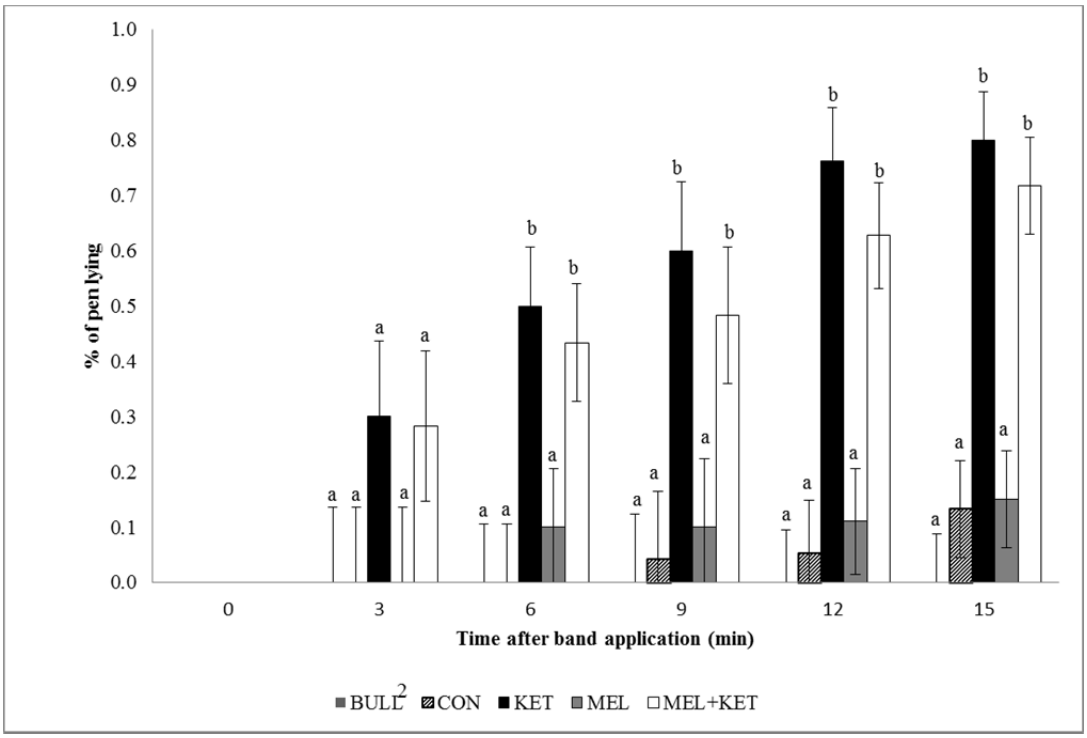
[1]BULL = sham procedure on d 0, CON = band on d 0, KET = band on d 0 with subcutaneous ket-stun, MEL = band on d 0 with oral meloxicam, MEL+KET = band on d 0 with subcutaneous ket-stun and oral meloxicam.
[2]CAST = Contrast between pooled CON, KET, MEL, MEL+KET means and BULL mean.
[3]INT = Interaction of main effects (KET and MEL).
[4]KET = Contrast between KET and non-KET treatments.
[5]MEL = Contrast between MEL and non-MEL treatments .

Table 3: Least square means (± SEM) for ADG, DMI, G:F, initial BW and final BW in response to castration and main effects of subcutaneous ket-stun and oral meloxicam after band castration or sham in weaned beef bulls

	Treatment[1]						Contrast, *P* =			
Item	BULL	CON	KET	MEL	KET+MEL	SEM	CAST[2]	INT[3]	KET[4]	MEL[5]
ADG, kg	1.60	1.14	1.28	1.17	1.27	0.10	<0.001	0.80	0.14	0.93
DMI, kg	4.96	4.69	4.44	4.82	4.76	0.38	0.34	0.71	0.55	0.38
G:F	0.14	0.06	0.10	0.08	0.11	0.02	0.02	0.60	0.12	0.53
Initial BW, kg	312.1	288.3	283.0	281.5	279.0	6.12	<0.001	0.81	0.51	0.36
Final BW, kg	338.4	294.6	291.3	291.1	296.3	7.72	<0.001	0.44	0.86	0.86

[1]BULL = sham procedure on d 0, CON = band on d 0, KET = band on d 0 with subcutaneous ket-stun, MEL = band on d 0 with oral meloxicam, MEL+KET = band on d 0 with subcutaneous ket-stun and oral meloxicam.
[2]CAST = Contrast between pooled CON, KET, MEL, MEL+KET means and BULL mean.
[3]INT = Interaction of main effects (KET and MEL).
[4]KET = Contrast between KET and non-KET treatments.
[5]MEL = Contrast between MEL and non-MEL treatments.

Figure 1: Least square means (± SEM) for post-castration observations collected at 3 min intervals for a 15-min period after sham procedure or banding with or without meloxicam or ket-stun in weaned beef bulls[1]



[1]BULL = sham procedure on d 0, CON = band on d 0, KET = band on d 0 with subcutaneous ket-stun, MEL = band on d 0 with oral meloxicam, and MEL+KET = band on d 0 with subcutaneous ket-stun and oral meloxicam.
[2]No animals in BULL treatment were observed lying during the observation period.
[ab] At each time point, means without common superscripts differ ($P < 0.05$).

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IMPACT OF MATERNAL NUTRITION AND POST-WEANING MANAGEMENT ON STEER AND HEIFER PROGENY IN A LATE SPRING CALVING SYSTEM

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ABSTRACT: An ongoing trial is being conducted to evaluate effects of winter supplementation on cow performance and effects of post-weaning management on steer and heifer progeny. Pregnant, May-calving, crossbred cows (n = 176; BW = 477 ± 69 kg) grazed dormant upland range or meadow from December 1 to February 28 and received 0 or 0.45 kg/d (DM) of 32% CP supplement in a 2 × 2 factorial arrangement. Calves were weaned January 1, blocked by BW and subsequently placed on 1 of 2 winter treatments: 1) graze winter meadow with 0.45 kg/d supplement, or 2) offered meadow hay (ad libitum) plus 1.81 kg/d supplement. One half of steer calves from each wintering system were then placed in a feedlot (calf-fed system) on May 15. The remaining steers and heifers grazed upland range until approximately August 30, when the steers were placed in a feedlot (yearling-fed system). Heifers were maintained in a single group. The first 2 yr of data are presented. Winter treatment did not affect ($P > 0.10$) cow BW or BCS at precalving, prebreeding, or weaning. Subsequent pregnancy rates were not influenced ($P = 0.60$) by winter treatment. Steers fed hay following weaning had increased ($P = 0.03$) ADG during winter compared with steers grazing meadow. In the yearling-fed system, hay-fed steers remained heavier ($P = 0.05$) through second implant, although final BW was similar ($P = 0.28$). Post-weaning management did not influence steer carcass data. Heifers managed on hay post weaning had greater ($P = 0.03$) ADG through winter treatment compared with meadow heifers; however, percent pubertal and pregnancy rates were similar ($P = 0.49$). Supplementation did not affect cow BW, BCS, or subsequent pregnancy rates. Post-weaning management affected calf BW from weaning through the treatment period; however, preliminary data indicate minimal effects of winter treatments on subsequent heifer pregnancy rate or steer feedlot and carcass characteristics.

Key words: beef cattle, supplementation, yearling systems

Introduction

The greatest variable cost associated with cow-calf production is feed (May et al., 1999). The amount of harvested and purchased feed required to sustain a Nebraska Sandhills cow herd can be reduced by calving late in the spring, better matching the cow's nutrient requirement with grazed forage (Adams et al., 1996; Clark et al., 2004). Altering the calving date may provide additional enterprise opportunities (stocker system) and market timing, which may be economically advantageous (Stockton et al., 2007). Shifting the calving date may also provide flexibility to sell calves at different ages and BW. (Griffin et al., 2012)

The nutritional requirements of a spring calving beef cow grazing dormant Sandhills range during late gestation typically exceed the nutrient content of the grazed forage (NRC, 2000). Protein is commonly supplemented to maintain cow BCS during winter grazing. Supplementing protein also increases weaning BW and the proportion of live calves at weaning (Stalker et al., 2006). Supplementing beef cows during late gestation has been shown to affect the lifelong productivity of the calf by altering postweaning growth, carcass composition, calf health in the feedlot (Larson et al., 2009), and heifer fertility (Martin et al., 2007).

Therefore, objectives of the current study are to evaluate the effects of winter supplementation while grazing dormant Sandhills winter range or meadow on cow performance and effects of post-weaning management on steer and heifer progeny in a late spring calving herd.

Materials and Methods

All animal procedures and facilities were approved by the University of Nebraska Institutional Animal Care and Use Committee.

Cow-calf management. An ongoing trial is being conducted utilizing composite Red Angus × Simmental cows and their progeny at the Gudmundsen Sandhills Laboratory (**GSL**), Whitman, and West Central Research and Extension Center (**WCREC**), North Platte. Cows grazed either dormant upland winter range or meadow from December 1 to March 29 and received 0 or 0.45 kg DM animal^{-1}·d^{-1} of a 32% CP supplement (Table 1). Supplement was delivered 3 times/wk on a pasture (35.6 ha) basis. Cows were managed as a common group the remainder of the year. Cows were estrous synchronized with a single injection of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, New York, NY) 5 d after being placed with bulls (1:20 bull to cow ratio) approximately August 1 for 45 d. Pregnancy was determined via rectal palpation or ultrasonography at weaning in early January. Cows were removed from the study for reproductive failure, calf death, or injury. Approximately 5 d post weaning, calves were placed on 1 of 2 winter treatments: graze winter meadow with 0.45 kg DM animal^{-1}·d^{-1} supplement (**MDW**), or offered meadow hay (ad libitum) and 1.81 kg DM animal^{-1}·d^{-1} supplement (**HAY**).

Table 1. Composition and nutrient analysis of supplement

Item	DM, %
Ingredient	
Dried distillers grains with solubles	62.0
Wheat middlings	11.0
Cottonseed meal	9.0
Dried corn gluten feed	5.0
Molasses	5.0
Calcium carbonate	3.0
Trace minerals and vitamins[1]	3.0
Urea	2.0
Nutrient	
CP	31.6
Undegradable intake protein, % CP	47.6
TDN	89.4

[1]Formulated to include 80 mg·0.45 kg monensin.

Heifer management. After January weaning, heifers were blocked by BW and assigned to either MDW or HAY treatment until May 15. Winter treatments were replicated twice. Following winter treatment, heifers were managed as a single group. Heifers were moved to upland range pastures for the breeding season. Two blood samples were collected 10 d apart prior to the breeding season to determine luteal activity. Heifers were considered pubertal if serum progesterone concentrations were >1 ng/mL. Heifers were estrous synchronized with a single injection of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, New York, NY) 5 d after being placed with bulls (1:20 bull to heifer ratio) on approximately July 25 for 45 d. Pregnancy was determined via transrectal ultrasonography in late October. Data reported was collected in 2011 (n = 65) and 2012 (n = 65).

Steer management. After January weaning, steers were blocked by BW and assigned to either MDW or HAY treatment. Winter treatments were replicated twice. On May 15 one-half of the steers from each winter treatment were placed in a feedlot at WCREC (calf-fed system). The remaining steers were implanted with Revalor G (40 mg trenbolone acetate and 8 mg estradiol, Merck Animal Health, Summit, NJ) and subsequently grazed upland summer range until approximately August 30, and then placed in the feedlot (yearling-fed system). Upon feedlot entry, steers were limit fed 5 d at 2.0% BW, weighed 2 consecutive d, and adapted (21 d) to a common finishing diet of 48% dry rolled corn, 40% corn gluten feed, 7% prairie hay, and 5% supplement. In the calf-fed system, Synovex Choice (100 mg trenbolone acetate and 14 mg estradiol benzoate, Ft. Dodge Animal Health, Overland Park, KS) was administered at feedlot entry and Synovex Plus (200 mg trenbolone acetate and 28 mg estradiol benzoate, Ft. Dodge Animal Health, Overland Park, KS) approximately 100 d later. In the yearling-fed system, Ralgro (36 mg zeranol, Merck Animal Health, Summit, NJ) was administered at feedlot entry, followed by Synovex Plus approximately 60 d later. Steers were slaughtered when estimated visually to have 1.3 cm fat thickness over the 12th rib. Steers were slaughtered at a commercial abattoir, and carcass data were collected after a 24 h chill. Final BW was calculated from HCW using a standard dressing percentage (63%). Data reported were collected in 2011 (n = 68) and 2012 (n = 54).

Statistical analysis. Cow and progeny winter treatments and steer feedlot treatment were applied on a pasture or group basis. Pasture (n = 4/yr) served as experimental unit for cow performance and reproductive data. Winter treatment (n = 4/yr) served as experimental unit for heifers. Winter treatment × feedlot treatment served as the experimental unit for the steers. Data were analyzed with the GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, NC). Model fixed effects for cow data included winter treatment and age. Winter treatment, feedlot system, and appropriate interactions ($P < 0.05$) were included in the progeny model. Year was considered a random effect for cow and calf variables.

Results and Discussion

Cow-calf results. Cows that grazed meadow with supplement had greater ($P = 0.03$) BW gain over the treatment period compared with cows grazing range without supplement (Table 2). Winter treatment did not affect ($P > 0.10$) BCS over the treatment period. Winter treatment also did not affect cow BW or BCS at precalving, prebreeding, or weaning ($P > 0.10$). Calf birth BW, calving difficulty, calf vigor, and subsequent pregnancy rates were not affected ($P > 0.15$) by supplementation or winter treatment. There was a difference of 21 percentage points (± 17 %) in pregnancy rates between the youngest (3-yr-old) cows compared with older cows despite a lack of significance (67 vs. 88%, $P = 0.24$), which may be a result of limited data at this point. Moving to a late-spring calving season moves the breeding season to late summer, coinciding with declining forage nutrient quality, which may have a greater impact on pregnancy rates in young growing cows (Rensiss and Scarmuzzi, 2003).

Heifer progeny results. The effects of winter management system on heifer progeny are presented in Table 3. Heifers on HAY treatment had greater ($P = 0.03$) winter ADG than MDW heifers and tended ($P = 0.10$) to have increased BW in May and July. Percent pubertal at the beginning of the breeding season and pregnancy rates were similar ($P > 0.39$) between treatments. Patterson et al. (1992) reported improved nutrition during the post-weaning to prebreeding phase allowed for successful breeding of yearling beef heifers, whereas decreased nutritional levels during post-weaning to prebreeding delayed first estrus and pregnancy rates of yearling heifers. Currently, heifers on HAY treatment have a numerically higher proportion of heifers pubertal prior to breeding (78 vs. 69%) and higher pregnancy rate (68 vs. 61%) compared with MDW heifers despite a lack of significance ($P = 0.39$). Again, this may be related to limited data. Pregnancy rates were numerically (approximately 20 percentage points) lower than pregnancy rates in March-born heifers on the same ranch (Larson et al., 2011), which may be a function of declining nutrient quality during the later breeding season. Younger cows and heifers may require supplemental

nutrition during the breeding season to achieve similar pregnancy rates as beef females in an earlier spring calving herd.

Steer progeny results. The interaction between winter treatment and feedlot system was not significant ($P > 0.10$). Therefore, only main effects of winter treatment and feedlot system will be presented (Table 4). Steers on HAY treatment had greater ($P = 0.03$) ADG compared with steers on MDW treatment during treatment period and tended ($P = 0.07$) to have increased BW at end of winter treatment in May. In the calf-fed system, steers on HAY treatment tended to have greater ($P = 0.06$) feedlot entry BW than steers on MDW treatment and tended ($P = 0.06$) to have greater BW at second implant in August. Winter treatment did not influence ($P > 0.10$) final BW or carcass characteristics in the calf-fed system (Table 4). In the yearling-fed system, steers on HAY treatment had greater ($P = 0.05$) BW entering the feedlot in September until time of second implant ($P = 0.02$) in November. Winter treatment had no effect ($P > 0.10$) on final BW or carcass characteristics in the yearling-fed system. At present, with 2 yr of data, steers from the calf-fed and yearling-fed systems have similar ($P \geq 0.34$) feedlot ADG and carcass characteristics.

Currently, winter management systems for cows or progeny have not had significant effects on subsequent dam or progeny performance. Additional data and economic analysis are required to make specific recommendations relating to management strategies for a late spring calving herd in the Nebraska Sandhills.

Implications

Cow and calf management strategies should be designed to complement available resources. As input prices continue to rise, beef producers will consider alternative strategies to reduce costs and potentially increase profits. A late spring calving season may reduce the need for supplementation of the cow herd during winter and provide additional management and marketing opportunities for progeny, potentially impacting profitability.

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Table 2. Effects of winter grazing treatment[1] on cow BCS, BW, pregnancy rate, and calf BW

Item	MNS	MS	RNS	RS	SE[2]	*P*-value
Cow BCS						
January	4.4	4.4	4.5	4.4	0.2	0.76
Winter change	-0.2	0.0	0.0	0.2	0.1	0.16
Pre-calving	4.5	4.6	4.8	4.8	0.2	0.31
Pre-breeding	5.3	5.4	5.4	5.4	0.1	0.81
Cow BW						
January BW, kg	448	453	450	447	9	0.97
Winter BW gain, kg	48[ab]	54[a]	34[b]	51[ab]	4	0.03
Pre-calving BW, kg	478	485	466	480	23	0.54
Pre-breeding BW, kg	490	499	499	500	15	0.87
Pregnancy rate, %	84	88	73	77	1	0.60
Calf BW						
Birth BW, kg	36	35	34	35	1	0.45
Pre-breeding BW, kg	101	97	97	102	3	0.47
Weaning BW, kg	198	197	192	199	4	0.58

[1]Treatments: MNS = grazed meadow without supplement, MS = grazed meadow and 0.45 kg 32% CP supplement, RNS = grazed winter range without supplement, RS = grazed winter range and 0.45 kg 32% CP supplement.
[a,b]Within a row, means without common superscript differ at $P < 0.05$.

Table 3. Effects of winter grazing treatment[1] on heifer progeny

	HAY	MDW[1]	SE	*P*-Value
Winter ADG[2], kg	0.69	0.38	0.02	0.03
May BW, kg	279	238	3	0.07
June BW, kg	311	279	4	0.12
July BW, kg	326	295	4	0.10
Summer ADG[3], kg	0.80	0.94	0.03	0.18
October BW, kg	370	342	4	0.12
October BCS	5.7	5.5	0.05	0.22
Pubertal, %	78	69	7.7	0.47
Pregnancy rate, %	68	61	3.6	0.39

[1]Winter grazing treatments: HAY = meadow hay (ad libitum) and 1.81 kg 32% CP supplement; MDW = grazed winter meadow and 0.45 kg 32% CP supplement.
[2]Calculated from January weaning date to end of winter treatment on May 15 (126 d).
[3]Calculated from removal of winter treatment on May 15 to July 14 (60 d).

Table 4. Effects of winter treatment[1] and feedlot system[2] on steer performance

	HAY		MDW			*P*-Value	
	Calf-fed	Yearling-fed	Calf-fed	Yearling-fed	SE	Winter treatment	Feedlot System
Winter ADG[3], kg	0.68	0.71	0.36	0.36	0.02	0.03	0.64
May BW, kg	289	295	252	248	5	0.07	0.86
Feedlot entry BW, kg	289	367	252	337	7	≤0.06	0.09
Feedlot ADG[4], kg	1.77	1.90	1.90	1.88	0.01	0.47	0.39
Final BW, kg	667	684	656	649	13	0.28	0.77
HCW, kg	420	431	413	409	7	0.28	0.77
Marbling score[6], kg	520	555	521	544	8.4	0.71	0.43
12th rib fat, cm	1.43	1.50	1.43	1.47	0.09	0.90	0.65
LM area, cm^2	95	96	93	92	3	0.41	0.94
Yield grade	3.17	3.36	3.25	3.35	0.12	0.83	0.43
USDA Choice, %	93	96	90	100.0	0.06	0.95	0.34
1,000 lb carcass, %	11	28	18	4	0.09	0.42	0.83

[1]Winter grazing treatments: HAY = meadow hay (ad libitum) and 1.81 kg 32% CP supplement; MDW = grazed winter meadow and 0.45 kg 32% CP supplement.
[2]Feedlot system: Calf-fed steers entered feedlot on May 15; Yearling-fed steers entered feedlot on August 30.
[3]Weaning (January) to end of winter treatment (May 15, 126 d).
[4]May 15 to Dec 11 (210 d) for calf -fed system and September 14 to February 28 (167 d) for yearling-fed system.
[6]Small[00] = 400.

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EFFECT OF SUPPLEMENTATION FREQUENCY ON PERFORMANCE OF SPRING-CALVING COWS SUPPLEMENTED WITH DRIED DISTILLERS GRAIN

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ABSTRACT: The objective of this study was to evaluate the effect of supplementation frequency of dried distiller's grains with solubles (**DDGS**) on performance of spring calving cows grazing dormant native range (7.5% CP, DM basis). Pregnant Angus-cross cows (n = 120; age = 5.4 ± 0.30 yr; BW = 563 ± 77.1 kg; BCS = 5.1 ± 0.04) were stratified by age, BW, and BCS and assigned randomly to one of three treatments in a completely randomized design: 1) daily supplementation (**DDGD;** control); 2) supplementation every 3 d (**DDG3**); 3) supplementation every 6 d (**DDG6**). Treatments were initiated 84 d prior to expected onset of calving. Supplement was prorated to supply 0.27 kg CP·head^{-1}·d^{-1} (0.82 kg DDGS·head^{-1}·d^{-1}). Cows were sorted daily before supplementation at 0830 h. Forage sorghum hay (6.9% CP, DM basis) was supplied at 50% of expected DMI to ensure ample intake and ease grazing pressure due to drought (annual precipitation was 81.5% of the National Weather Service 30-yr average). Post-calving, cows were fed DDGS daily until turnout on summer pasture (d 132). Cow BW and BCS were measured every 28 d prior to supplementation, within 12 h of parturition, and on d 132. Proportion of cows consuming hay 60 min post-feeding was observed during an 11 d period. Progesterone (**P4**) concentrations were measured in paired serum samples collected before ovulation synchronization initiation to determine proportion of estrual cows. Supplementation frequency did not affect final BW and BCS (P = 0.95 and 0.37, respectively) or BW and BCS change (P = 0.82 and 0.74, respectively). A greater proportion (P = 0.01) of DDGD, DDG3 or DDG6 (not supplemented on the d of observation) were consuming hay 60 min post-feeding compared to DDG6 (supplemented on the d of observation). No differences (P > 0.05) were observed for calf BW at birth or weaning or calf ADG. Supplementation frequency did not affect (P > 0.50) proportion of estrual cows at initiation of ovulation synchronization (37%), first service conception rate (69%) or final pregnancy rate (96%). Reducing supplementation frequency to every 6 d did not adversely affect performance or reproductive success of spring-calving beef cows supplemented with DDGS.

Key Words: Cow performance, dried distillers grains, supplementation frequency

Introduction

During the winter cattle commonly graze standing dormant native range (< 6% CP). The lack of dietary protein provided by this dormant forage limits cow performance. Thus it is vital that cattle grazing dormant native range be offered supplemental protein to sustain BW and BCS during the last trimester of gestation (Heldt et al., 1999; Mathis and Sawyer, 2007; Olson and Harty, 2007). Protein supplementation improves cow BW and BCS, and has been shown to increase calf BW at birth (Farmer et al., 2001, Mathis, 2003). The production responses associated with protein supplementation occur not only from the additional protein provided by the supplement, but a more efficient utilization of the dormant forage base (Bohnert et al., 2002a).

Protein supplementation burdens cattlemen with extra cost, but reducing supplementation frequency may be used to reduce delivery costs (Mathis and Sawyer, 2007;). Supplementing a high protein feedstuff (> 30% CP) as infrequently as once per week has resulted in similar BW and BCS changes compared to daily delivery (Huston et al., 1999; Bohnert et al., 2002b; Schauer et al., 2005).

Dried distiller's grains with solubles (**DDGS**) have become an increasingly popular feedstuff in beef cattle production systems. The nutrient profile, availability, and cost of DDGS make it a suitable substitute for conventional protein feedstuffs. Morris and coworkers (2005) observed a 0.12 kg increase in ADG with every 0.45 kg of DDGS supplemented to cattle fed low-quality hay. Recently, cattle fed DDGS exhibited lower BW and BCS loss while grazing dormant native range compared to a wheat middlings/cottonseed meal supplement (Winterholler et al., 2012).

Therefore the objective of this study was to evaluate the effects of supplementation frequency on cow performance, reproductive success, eating behavior, and subsequent calf performance of spring-calving cows fed DDGS.

Materials and Methods

Animal care practices used in this study were approved by the
Kansas State University Institutional Animal Care and Use Committee (Protocol no. 3175)

Animals and Experimental Design. Pregnant Angus-cross cows (n = 120; age = 5.4 ± 0.30 yr; initial BW = 563 ± 7.0 kg; initial BCS = 5.1 ± 0.04) were maintained on common native range (7.5% CP, DM basis; Table 1) for 84 d. Composition of the range site was determined using a step point method (Evans and Love, 1957). The range site was comprised of the following species; Sideoats Grama

(*Bouteloua curtipendula*), Western Wheatgrass (*Agropyron smithii*), Blue Grama (*Bouteloua gracilis*), Japanese Brome (*Bromus japonicus*), and Buffalograss (*Bouteloua dactyloides*) (K. Harmoney, 2011, Kansas State University Agricultural Research Center – Hays, 1232 240th Ave, Hays, KS, personal communication).

Cows were stratified by age, BW, and BCS and assigned randomly to one of three supplement treatments: 1) DDGS daily (**DDGD;** control); 2) DDGS every 3 d (**DDG3**); 3) DDGS every 6 d (**DDG6**). Dried distiller's grains with solubles (29.5% CP, DM basis; Table 1) originated from a single location, was delivered and stored in bulk for use throughout the treatment period. Cows were sorted daily into treatment groups and supplement was delivered approximately at 0830 h into a bunk for consumption. Only one set of bunks was available, therefore on days when multiple supplement treatments were fed each group would be given ample time to finish the supplement before being moved out of the feeding area. Cows were allotted 71.1 cm of linear bunk space/head. Supplement was offered to supply 0.27 kg CP·head^{-1}·d^{-1} (0.82 kg DDGS·head^{-1}·d^{-1}). For example, cattle receiving supplement once every 6 d received 1.62 kg CP/head (4.92 kg DDGS/head) on the day of supplementation.

Forage sorghum hay (6.9% CP, DM basis; Table 1) was supplied at 50% of expected DMI to ensure ample intake and ease grazing pressure due to drought (annual precipitation was 81.5% of the National Weather Service 30-yr average). Mineral (Suther's Prairie Cow 4P; Suther's Feeds, Frankfort, KS) was available ad libitum before, during, and after the experiment. At the onset of calving, treatments were discontinued and cows were fed DDGS daily in a common pasture and fed ad libitum forage sorghum hay. Cows were maintained in this manner until turnout on summer pasture (d 132).

Table 1. Nutrient composition (DM basis) of native range, dried distillers grains with solubles (DDGS) and forage sorghum hay (SH).

	Nutrient Composition		
Item	Native Range	DDGS	SH
DM, %	74.43	89.24	86.61
CP, %	7.49	29.53	6.93
NE_m, Mcal/kg	0.71	1.94	1.06
NE_g, Mcal/kg	0.18	1.21	0.51
NDF, %	59.18	30.39	59.30
ADF, %	44.43	17.08	36.89
TDN, %	42.57	76.59	52.75
Calcium, %	0.58	0.08	0.49
Phosphorus, %	0.11	0.80	0.15
Sulfur, %	0.09	0.43	0.10

Data Collection. Range forage samples for nutrient analysis were obtained prior to trial initiation. Samples were collected from multiple 1 m^2 areas, clipped 2 cm above the surface, placed in plastic sealable bags, and immediately frozen. All samples were thawed and passed through a Wiley Mill (2 mm screen; Arthur H. Thomas, Philadelphia, PA), composited, and frozen at -20°C until laboratory analysis for nutrient content. Core samples (n = 20) were taken from a random sample of forage sorghum bales, composited, and frozen. Random samples of DDGS were taken at delivery, composited, and frozen. Feed samples were submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) and analyzed for DM, CP, NE_m, NE_g, NDF, ADF, TDN, Ca, P, and S.

Cow BW and BCS were measured every 28 d, immediately following parturition (within 12 h), and on d 132 (summer turnout). Cows were weighed approximately at 0900 h. Supplement and hay were withheld the morning of BW collection and fed immediately after all cows had been weighed. Two independent, qualified observers assigned BCS using a 9-point scale (1= extremely emaciated, 9=extremely obese; Wagner et al., 1988) on each weigh date, including the day of parturition. Longissimus dorsi muscle (LM) depth, 12-th rib fat thickness, and marbling score were measured ultrasonically on d 0 and 84 using an Aloka 500V (Aloka Co., Ltd., Wllingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-12mm window). Ultrasound images were collected with Cattle Performance Enhancement Company (CPEC, Oakley, KS) software. Backfat thickness, LM depth and marbling score were estimated with procedures that incorporated image analysis software (Brethour, 1994) that are an integral component of the CPEC software. Calf BW was obtained within 12 h of birth, on d 132, and at weaning (d 196). Calves were weaned at 113 ± 17 d of age due to the persistence of drought conditions during the grazing season.

Eating Behavior. For an 11-d period, cow eating behavior was observed. Cows were fed hay approximately at 0930 h each day. The number of cows, within each treatment group, actively consuming hay was recorded 60 min post-feeding each day during the 11-d observation period.

Estrus Determination. Blood samples were collected via jugular venipuncture 10 d before and on the day ovulation synchronization was initiated. Samples were collected into 10 mL serum vacutainer tubes (BD Vacutainer™, Becton, Dickinson, and Company, Franklin Lakes, NJ) then immediately placed on ice, allowed to clot for 24 h at 4°C and then centrifuged (1,500 × g) for 10 min. Serum was decanted into 12 × 75 mm plastic tubes, capped and immediately frozen (-20°C). Concentration of progesterone (**P4**) in serum was subsequently quantified using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA; Stevenson, 2011). Intra- and interassay CV were 3.4 and 7.6% respectively and sensitivity of the assay was 0.009 ng/mL. Blood collected 10 d before and on the d ovulation synchronization was initiated was used to verify the functional presence of a corpus luteum. If any 1 of the 2 samples contained P4 >1 ng/mL (typical of cows in the luteal phase of the estrous cycle), cows were assumed to be cycling before the onset of ovulation synchronization

treatments. If concentrations in the 2 samples were <1 ng/mL, cows were considered to be noncycling.

Ovulation Synchronization and Breeding. Ovulation was synchronized in equal proportions of the three supplementation treatments by assigning cows from each treatment randomly to the 7 d Co-Synch + controlled internal drug release (**CIDR**; EAZI-Breed CIDR®, Pfizer Animal Health, New York, NY) protocol (Larson et al., 2006) or a modification of the 7 d Co-Synch + CIDR protocol. Cows assigned to the 7 d Co-Synch+CIDR protocol received 100 µg of GnRH intramuscularly (10 d before fixed time AI; 2 mL of Cystorelin; Merial, Duluth, GA) and a CIDR (EAZI-Breed CIDR containing 1.38 g of P4) insert followed in 7 d by an injection of 25 mg prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) intramuscularly (3 d before fixed time AI; 5 mL of Lutalyse; Pfizer Animal Health) and the CIDR was removed followed in 62 h by fixed-time AI (**FTAI**) and a second 100 µg injection of GnRH. Cows assigned to the modified 7 d Co-Synch+CIDR protocol were treated similarly but also received an injection of 25 mg $PGF_{2\alpha}$ intramuscularly 20 d before FTAI and an injection of 100 µg GnRH intramuscularly 17 d before FTAI. Cows were exposed to fertile bulls 10 d after FTAI for the remainder of the 45-d breeding season.

Thirty-five d after artificial insemination, first service conception rate (**FSCR**) was determined by transrectal ultrasonography (Aloka 500V, 5MHz transrectal transducer, Wallingford, CT). A positive pregnancy outcome required the presence of uterine fluid and an embryo with a heartbeat. Final pregnancy rate (**PR**) was determined 35 d after the end of the breeding season via transrectal ultrasonography.

Statistical Analysis. Performance data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Initial BW, BW at parturition, BW change, initial BCS, BCS at parturition, BCS change, calf BW at birth, calf BW at weaning, calf ADG from birth to weaning and average calving date were used as the dependent variables. Cow was utilized as the experimental unit. The number of cows consuming hay 60 min post-feeding was converted to proportion within supplement frequency treatment group. Data were transformed, due to non-normality, using the arcsine-square root (angular) transformation according to Martin and Bateson (1986). Behavior data were analyzed using the GLM procedure of SAS using supplement-frequency treatment group, supplementation status (supplemented or non-supplemented on the day of observation) and their interactions were used as independent variables. Behavior data are reported as proportion of supplement-frequency treatment group. Reproductive data were analyzed using the CATMOD procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the main effects of supplementation frequency, ovulation synchronization method and their interaction. Reproductive success among supplement-frequency treatment groups was not affected ($P > 0.50$) by ovulation synchronization method and it was removed from the model for final analysis. Least square means are presented and differences were considered significant at $P \leq 0.05$.

Results and Discussion

Initial cow BW and BCS were not different among treatments ($P > 0.05$; Table 2). Supplementation frequency did not affect final cow BW or BCS ($P = 0.95$ and 0.37, respectively). As a result, the overall change in BW and BCS were not different ($P = 0.82$ and 0.74, respectively). Other researchers (Beaty et al., 1994; Schauer et al., 2005) found similar results utilizing high-RDP supplements. These data suggest that infrequent supplementation of a high-RUP feedstuff can result in cow performance similar to traditional supplements. Cow BW at summer turnout was similar among treatments ($P = 0.80$). Average calving date, calf BW at birth, weaning, and ADG were not affected ($P > 0.20$) by supplementation frequency. Beaty et al. (1994) also reported similar calf performance among infrequently supplemented cows.

The first limiting nutrient for cattle grazing dormant native range is typically RDP (Bohnert et al., 2002b; Winterholler et al., 2012). Therefore, the use of high-RDP supplements such as soybean and cottonseed meal has become common supplements in winter feeding systems. The RDP fraction of DDGS is lower than typical supplements (40-50%; Shurson and Noll, 2005). Atkinson et al. (2010) found that lambs supplemented with a 50:50 RDP/RUP mixture had improved total ruminal OM digestibility compared to high-RDP supplementation. This relative split between RDP and RUP provides adequate nitrogen to the rumen to stimulate microbial efficiency and urea recycling, plus increased immediate protein utilization by the host animal compared to traditional high-RDP supplements (Atkinson et al., 2010). Bohnert et al. (2002b) theorized that high-RUP feedstuffs were more conducive to infrequent supplementation through increased deamination of free AA by the liver and a lower ruminal ammonia concentration on non-supplementation days which increases N recycling to the gut. Cows receiving DDGS once every 6 d in the current study may have compensated for a lack of daily protein through this mechanism, resulting in similar performance to those cows supplemented daily.

There was a significant difference ($P = 0.01$) between supplementation frequency-supplementation status groups (Fig. 1) for the proportion of cows consuming hay 60 min post-feeding. These data indicate that the amount of supplement provided may affect cow eating behavior. However, the decreased number of cows consuming hay on the day of supplementation did not affect performance as measured as measured by BW, BCS, or reproductive success. Conversely, although a larger proportion of cows were not consuming hay in some supplementation treatment groups they may have consumed more standing dormant grass.

Proportion of cows considered to be estrual at initiation of ovulation synchronization was not different ($P = 0.86$; Table 3) between supplementation frequency treatments and averaged 37.0%. Frequency of late gestation supplementation did not affect first service conception rate (68.5%; $P = 0.90$) or final pregnancy rate (96.3%; $P = 0.55$).

Under the conditions of our study, reducing the frequency of DDGS supplementation to as little as once

every 6 d did not adversely affect cow and calf performance, or subsequent reproductive success.

Implications

The economic benefits associated with reducing supplementation frequency of DDGS make this feeding system a viable option for cattle producers seeking to reduce production costs without adversely affecting performance. The reduction in labor and fuel costs realized may aid producers in maintaining the long-term sustainability of a cow-calf enterprise in a high cost environment.

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Table 2. Performance of cows receiving dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3) or every 6 d (DDG6).

	Treatment				
Item	DDGD	DDG3	DDG6	SEM	*P*-value
Number of cows	38	31	37		
Cow BW, kg					
Initial	563.2	569.9	562.3	7.39	0.91
Calving	563.9	569.9	565.6	7.57	0.95
Change	0.7	0.0	3.3	2.22	0.82
Turnout[1]	595.5	602.9	590.2	7.48	0.80
BCS					
Initial	5.07	5.18	4.97	0.05	0.23
Calving	5.28	5.31	5.16	0.04	0.37
Change	0.21	0.13	0.19	0.04	0.74
Avg. Calving Date	03/24/2012	03/22/2012	03/22/2012	0.44	0.20
Calf BW, kg					
Birth	38.4	39.4	37.8	0.97	0.33
Weaning[2]	147.3	152.9	153.0	3.48	0.23
Calf ADG, kg/d	1.00	1.05	1.05	0.03	0.31

[1] Weight at turnout onto summer pasture (d 132)
[2] Calves were weaned at 113 ± 17 d of age

Table 3. Reproductive performance of cows supplemented with dried distiller's grains with solubles daily (DDGD), every 3 d (DDG3) or every 6 d (DDG6).

	Treatment			
Item	DDGD	DDG3	DDG6	*P*-value
Estrual cows, %	39.5	33.3	37.8	0.86
FSCR[1], %	65.8	69.7	70.3	0.90
PR[2], %	100.0	97.0	91.9	0.55

[1] First service conception rate
[2] Final pregnancy rate

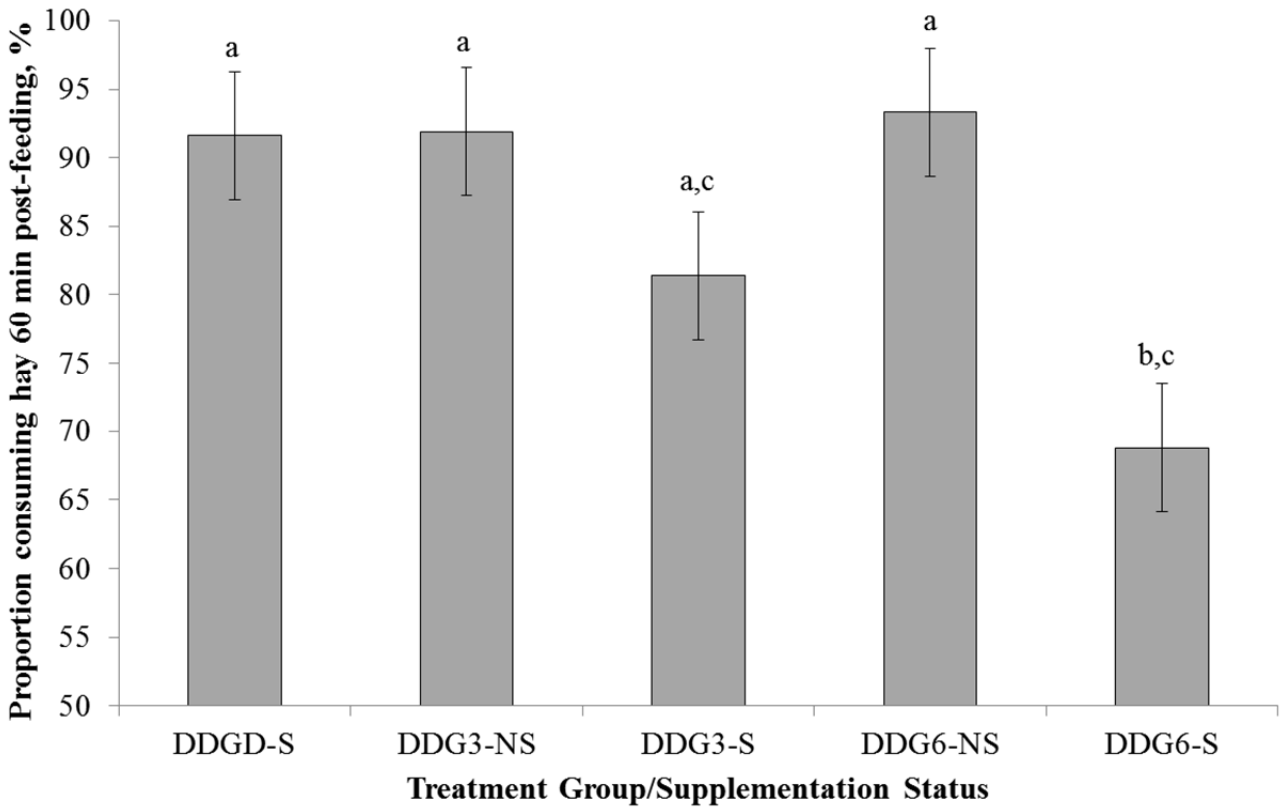

Figure 1. Proportion of supplementation-frequency treatment group (dried distiller's grain daily = DDGD; dried distiller's grain every 3 d = DDG3; dried distiller's grain every 6 d = DDG6) and supplementation status on day of observation (supplemented = S; non-supplemented = NS) consuming hay 60 min post-feeding during an 11 d observation period during the 84-d supplementation frequency experiment. [a,b,c,d] Bars without a common superscript differ (supplementation-frequency × supplementation status interaction; $P = 0.01$).

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THE EFFECTS OF WINTER PROTEIN SUPPLEMENTATION DURING THE THIRD TRIMESTER ON COW AND SUBSEQUENT CALF PERFORMANCE

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ABSTRACT: Angus based cows (n=120) were randomly allocated to 1 of 20 pens (10 pens/treatment; 6 cows/pen) and assigned to 1 of 2 dietary treatments to determine the effects of protein supplementation during the third trimester on cow and subsequent calf performance. Cows had ad libitum access to grass hay (6% CP; 53% TDN) and offered either: 1) 0.91 kg/d DDGS based protein supplement (30% CP; **PROTEIN**) or 2) no protein supplement (**NOPROTEIN**). The cows were weighed and BCS on consecutive days at the beginning, middle, and end of the experiment. Subsequent progeny performance was measured. Data were analyzed using the MIXED procedure of SAS with mean separation using LSD and considered significant when $P \leq 0.05$. Cow initial BW and BCS did not differ ($P \leq 0.57$) between treatments. However, cows on PROTEIN treatment had greater ($P \leq 0.04$) mid BW. BCS and final BW and tended ($P = 0.06$) to have greater final BCS than cows on NOPROTEIN treatment. Similarly, cows on PROTEIN treatment had greater BW gain ($P < 0.01$) and BCS change ($P = 0.02$). Subsequent pregnancy rates of cows were similar ($P = 0.46$) between treatments. Calf birth BW did not differ ($P = 0.15$) between treatments. However, weaning BW of calves born to PROTEIN dams was greater ($P = 0.03$) than NOPROTEIN. Heifer progeny BW and BCS at beginning of breeding season did not differ ($P \geq 0.44$) due to dam treatment. There was a trend ($P = 0.14$) for pregnancy rates of heifers born of PROTEIN dams to be greater (95% vs. 79% for PROTEIN and NOPROTEIN respectively) with a 16% greater response compared with heifers born of NOPROTEIN dams. Although there was a trend ($P = 0.16$) for steers born from PROTEIN dams to have a greater HCW ($P = 0.16$), feedlot performance due to dam treatment was similar. Dam treatment did not ($P \geq 0.18$) affect DMI, ADG, F:G, G:F, residual feed intake, yield grade, quality grade, LM area, marbling score, 12th rib fat, or KPH fat of steer progeny. Late gestation supplementation may impact subsequent progeny and further reinforces the importance of adequate nutrition during the final trimester of gestation.

Key words: fetal programming, gestation, nutrition

Introduction

Beef cows typically graze low-quality forage (<6% CP, DM basis) in the Northern Plains of the United States from late summer through winter and typically require protein supplementation to maintain or increase cow BW and BCS (Clanton and Zimmerman, 1970). Protein supplementation of dams grazing dormant forage during late gestation can increase progeny weaning BW, and fertility of heifer progeny (Stalker et al., 2006; Martin et al., 2007), while also improving the value of steer progeny at slaughter (Larson et al., 2009).

At present, little is known about the underlying mechanisms whereby alterations in conceptus nutrient intake result in permanent changes in structure, physiology, and metabolism of the neonate, a condition referred to as "fetal programming". Offspring of all livestock species, including cattle, born at below-average BW have a decreased probability of survival, conception rate, and growth (Funston et al., 2010). Fetal growth progresses most rapidly during the final trimester and up to 75% of growth occurs during the last 2 mo of gestation, thus nutrition during this time is crucial (Robinson et al., 1977).

Although studies have demonstrated protein supplementation during late gestation can enhance offspring development, detailed studies are needed to determine if changes occur in potential nutrient transport during pregnancy. Additionally, a substantial number of cattle are produced in the northern Great Plains, a region that is either too high in altitude or receives inadequate moisture to support corn production or corn stalk grazing as a winter feed source. Our hypothesis was that allowing gestating beef cows to consume low-quality harvested grass hay during late gestation without supplementation would affect offspring performance. Specifically, our objectives were to evaluate the impacts of maternal protein supplementation of irrigated meadow grass hay typical to the Intermountain West during the last trimester on: 1) cow BW and BCS, 2) calf birth and weaning BW, 3) conception rates of heifer offspring, and 4) feedlot performance of steer offspring.

Material and Methods

All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee.

Animals and treatments. One-hundred twenty mature, Angus-based crossbred cows from the Laramie Research and Extension Center were randomly assigned to receive either 0.91 kg/d of a DDGS based pelleted protein supplement (30% CP; **PROTEIN**) or 2) no protein supplement (**NOPROTEIN**) during late gestation. All cows were offered ad libitum low quality grass hay (6% CP; 53% TDN) from December 1 through February 28, 2011. Cows were randomly allocated to 1 of 20 pens, allowing for 10 replicates of each treatment with 6 cows per pen. Two-day cow BW was measured and BCS was determined by 3

independent evaluators at the initiation, midpoint, and conclusion of the study. Birth dates and birth BW of subsequent offspring was recorded following parturition and calf weaning BW was recorded on Sep. 3, 2011.

Prior to weaning all calves grazed with their dams and were managed similarly. Following weaning (~205 days of age), all heifer progeny were managed as a common group where they grazed the same Wyoming range until weather or forage quantity dictated that forage be supplemented. Individual 2-d BW and BCS were taken at the time of breeding and pregnancy rates were recorded.

All steer progeny were transported to the Sustainable Agriculture Research and Extension Center near Lingle, WY, and were managed as a common group. Each steer was equipped with an electronic identification device and had access to a GrowSafe (model 4000E, GrowSafe Systems Ltd., Airdrie, AB, Canada) system to determine feed intake. Feed intake was calculated by measuring the amount of feed offered and refused daily. Predicted DMI was determined by regressing ADG and metabolic midweight on actual feed intake (Cammack et al., 2005). Residual feed intake was calculated as the actual DMI minus the predicted DMI. Therefore, a more efficient steer has a negative RFI (observed feed intake less than predicted feed intake) and less efficient animal has a positive RFI (observed feed intake greater than predicted intake). Weights were measured on d 1, 45, and 90 of the study. Feed efficiency measures such as F:G and G:F were calculated as well as ADG for all steers on trial. All steers were slaughtered at Cargill Meat Solutions plant in Ft. Morgan, CO. where carcass data was collected including; HCW, marbling, 12th rib fat, LM area, quality grade, and yield grade.

Statistical analysis. All data were analyzed using the MIXED procedure of SAS (version 9.2, SAS Institute, Inc., Cary, NC). Cows were randomly assigned to pens and treatments. Cow performance data were analyzed with treatment as a fixed affect within the model and pen as the experimental unit. Subsequent calf performance was analyzed with treatment as a fixed effect within the model and individual calf as the experimental unit. Means were separated using LSD and considered significant when $P \leq 0.05$, tendency when $P < 0.10$, and a trend when $P < 0.15$.

Results and Discussion

By design, initial cow BW and BCS did not differ ($P \geq 0.57$) due to treatment (Table 1). However, cows receiving supplemental protein in late gestation had greater ($P \leq 0.04$) mid BW and BCS. Similarly, final BW was greater ($P = 0.04$) and final BCS tended to be greater ($P = 0.06$) for PROTEIN cows. Likewise, PROTEIN cows had greater overall BW gain ($P = 0.001$) and greater BCS change ($P = 0.02$) over the duration of the study. These supplementation effects were expected, as supplemented cows consumed more nutrients and were able to more readily surpass nutrient requirements necessary for fetal growth and gain maternal tissue during later stages of pregnancy.

All cows in the current study were fed at a similar level to meet requirements following parturition. Although PROTEIN cows had a slightly greater BCS prior to calving (0.15 greater), rebreeding pregnancy rates of PROTEIN cows did not differ ($P = 0.46$) compared with NOPROTEIN cows. These findings agree with earlier results reported by Engel et al., (2008), where there was no effect of DDGS supplementation in late gestation on pregnancy in heifers. Stalker et al., (2006), reported no improvement in pregnancy rates of mature beef cows grazing dormant upland range supplemented with protein during late gestation compared to a non-supplemented control, despite the grazed forage being deficient in CP and energy and unable to support nutrient requirements (NRC, 1996).

Calves born from PROTEIN cows were approaching a tendency ($P = 0.15$) for greater birth BW and had greater ($P = 0.03$) weaning BW compared with calves born to NOPROTEIN dams (Table 2). Recent studies have reported protein supplementation during late gestation did not affect calf birth BW (Martin et al., 2007). However, other evidence suggests that nutrient intake of the dam during gestation has the potential to alter birth BW of their offspring (Wiltbank et al., 1962) and potentially alter fetal muscle growth (Larson et al., 2009). Alterations in fetal growth may explain the trend for greater birth BW and the observed improvement in calf weaning BW. Although milk production was not measured in this experiment, it is unlikely that the 0.15 BCS difference in treatments prior to calving would biologically impact the cow. This explanation agrees with Lake et al., (2006), who reported similar milk production between cows that were managed to be a BCS 6 and BCS 4 at calving and fed to meet their respective requirements after calving.

There was no difference in heifer progeny BW ($P = 0.52$) or BCS ($P = 0.44$) at breeding due to dam treatment. Heifer pregnancy rates were not affected by dam treatment ($P = 0.14$) but approached a tendency where heifer calves born from PROTEIN dams showed a 16% greater response numerically (95% vs. 79% for PROTEIN and NOPROTEIN, respectively). This suggests our findings agree with reports from previous studies. Martin et al., (2007), observed heifers born to protein supplemented dams during the last one-third of gestation had increased pregnancy rates compared with heifers born to non-supplemented dams which may be related to age at puberty. Funston et al., (2008), reported heifers born to non-supplemented dams were less likely to attain puberty by the first breeding season compared with heifers from supplemented dams. Furthermore, previous research has also demonstrated heifers that have experienced 2 or 3 estrous cycles prior to the initiation of their first breeding season will have greater fertility than heifers inseminated on their first cycle (Byerley et al., 1987). The ability to conceive early will allow replacement heifers to calve earlier in the calving season likely resulting in greater lifetime cow production (Lesmeister et al., 1973). Our results lend support to the hypothesis that late gestation protein supplementation to cows consuming low quality forage diets has a positive effect on subsequent heifer reproductive potential.

Carcass characteristics of steer progeny was not affected ($P \geq 0.16$) by dam treatment in the current study (Table 3). Feed efficiency was also not ($P \geq 0.19$) affected due to dam supplementation. This is contrary to Underwood et al.,

(2008), which concluded that steers born to dams that grazed high quality pasture gained more BW during the finishing period, were heavier at slaughter, and had greater HCW compared to steers born to dams that grazed native range. Additionally, steers from cows that grazed improved pasture had carcasses with a greater percentage of body fat at slaughter and steers born from protein-supplemented cows had greater marbling scores with a greater proportion of steers grading USDA Choice compared to steers from non-supplemented cows (Larson et al., 2009; Stalker et al., 2006). This increase in percentage of steers grading USDA Choice without a substantial increase in yield grades may translate into an economic advantage for feedlot steers born to protein-supplemented dams during late gestation. The lack of detectable dam treatment effects on steer progeny may be partially due to the greater CP content of the forage offered in the current study compared to the CP described in Larson et al., (2009).

Beef cattle are often produced in systems that are deficient in nutrients such as crude protein. Protein supplementation during late gestation may be a crucial factor for both cow performance and future beef production.

Implications

Supplemental protein during late gestation to cows that are consuming grass hay common to the Intermountain West has the potential to improve cow and subsequent calf performance and enhance the future reproductive performance of heifer calves.

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Table 1. Effects of late gestational protein supplementation on cow performance

Item	PROTEIN [1]	NOPROTEIN [2]	SEM	*P*-value
Cow BW, kg				
Initial	562	553	11	0.57
Mid	609[a]	576[b]	11	0.04
Final	665[a]	631[b]	11	0.04
BW Change[3]	103[a]	77 [b]	3	< 0.01
Cow BCS				
Initial	4.57	4.57	0.05	0.99
Mid	4.86[a]	4.44[b]	0.06	< 0.01
Final	5.03	4.88	0.06	0.06
Rebreeding pregnancy rate, %	86	91	-	0.46

[a, b] Within an item, means differ ($P < 0.05$).
[1] PROTEIN cows received 0.91 kg/d of protein supplement during late gestation.
[2] NOPROTEIN cows did not receive any protein supplement during late gestation.
[3] Overall BW change.

Table 2. Effects of late gestational protein supplementation on subsequent heifer performance

Item	PROTEIN [1]	NOPROTEIN [2]	SEM	*P*-value
Calf birth BW, kg	38.5	37.1	0.69	0.15
Calf weaning BW, kg[3]	273[a]	261[b]	3.97	0.03
Calf age, d	165	167	1.87	0.64
Heifer BW at breeding, kg	348	343	6.47	0.52
Heifer BCS at breeding	5.33	5.24	0.09	0.44
Heifer pregnancy rate, %	95	79	-	0.14

[a, b] Within an item, means differ ($P < 0.05$).
[1] PROTEIN heifers born to cows that received 0.91 kg/d of protein supplementation during late gestation.
[2] NOPROTEIN heifers born to cows that did not receive any protein supplementation during late gestation.

Table 3. Effects of late gestational protein supplementation on subsequent steer feedlot and carcass characteristics

Item	PROTEIN[1]	NOPROTEIN[2]	SEM	*P*-value
Calf birth BW, kg	38.5	37.1	0.69	0.15
Calf weaning BW, kg[3]	273[a]	261[b]	3.97	0.03
Calf age, d	165	167	1.87	0.64
ADG, kg	1.77	1.83	0.08	0.55
DMI, kg	11.1	11.2	0.35	0.86
F:G, kg/kg	2.90	2.83	0.11	0.66
G:F, kg/kg	0.07	0.07	< 0.01	0.69
RFI, kg/d	-0.19	0.22	0.22	0.19
Hot Carcass Weight, kg	350	338	6.47	0.16
Yield Grade	3.12	3.12	0.14	0.97
Quality Grade[3]	17.5	17.7	0.20	0.45
Marbling[4]	593	602	19.77	0.74
12th rib fat thickness, cm	1.35	1.37	0.08	0.97
LM Area, cm^2	32.1	31.2	0.61	0.29

[1] PROTEIN steers born to dams that received 0.91 kg/d of protein supplementation during late gestation.
[2] NOPROTEIN steers born to dams that did not receive any protein supplementation during late gestation.
[3]Quality Grade: 15 = Select, 16 = Select$^+$, 17 = Choice$^-$, 18 = Choice^0, 19 = Choice$^+$.
[4]US marbling scores where 400 = Small^{00}, 500 = Modest^{00}, 600 = Slightly Abundent^{00}.

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EFFECT OF COBALT SUPPLEMENTATION ON RUMEN FERMENTATION AND BLOOD METABOLITES

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ABSTRACT: Thirty heifers (average BW = 256 ± 28.8 kg) were used in a completely randomized design to evaluate the influence of Co supplementation on rumen fermentation, blood metabolites, and total tract digestibility in calves. We hypothesized that supplemental Co would improve rumen fermentation and heifers would be less susceptible to stress. At weaning calves were assigned to treatment and pens (2 pens per treatment) for 27 d (back grounding phase; **BP**). Heifers were allowed *ad libitum* access to a forage diet (13.3% CP and 55.4% NDF; DM basis) for the duration of the experiment. Treatments included: 1) 0 mg Co heifer^{-1}·d^{-1} (**CON**), 2) 12.5 mg Co from $CoSO_4$ heifer^{-1}·d^{-1} (**SO4**) and 3) 12.5 mg Co from cobalt glucoheptonate heifer^{-1}·d^{-1} (**COPRO**). All treatments were pelleted using fine ground corn as a carrier and CON was fine ground corn only. Treatments were fed at a rate of 134.6 g hd^{-1}d^{-1}, 110.5 g hd^{-1}d^{-1}, and 128.1 g hd^{-1}d^{-1} for CON, SO4, and COPRO respectively. On d 28, heifers were transported 795 km to a holding facility, overnighted and returned to the originating location the following morning (stress phase; **SP**). Blood and ruminal fluid were collected prior to shipping (**SPBASE**) and upon arrival (**SPARR**) at the holding facility. Upon return to Las Cruces, blood was collected as heifers were unloaded and ruminal fluid was collected the next morning after calves were allowed access to the basal diet (**SPFINAL**). On d 32, heifers remained on BP treatments and were randomly assigned to individual pens for 15 d (post stress phase; **PSP**). Ruminal fluid and blood were sampled d 14 and fecal samples were collected on d 10 to 14 of PSP. For SPBASE, total VFA was higher (P = 0.01) for COPRO and SO4 than CON and did not differ for SPARR and SPFINAL (P > 0.40). During SPARR, acetate differed by Co source (P = 0.01). Ruminal Co concentration in SPBASE was greater for Co supplemented than CON (P < 0.01) and COPRO was higher than SO4 (P < 0.01). During PSP, ruminal ammonia tended to be highest for COPRO (P = 0.08), total VFA was greatest for COPRO and lowest for CON (P =0.01). Cortisol and haptoglobin tended (P = 0.06) to be lower for Co treated calves than CON. Total tract organic matter and NDF digestibility did not differ (P > 0.85) during PSP. Supplementing Co increased total VFA production while not changing total tract digestibility and thereby may enhance energy availability to stressed heifers.

Keywords: beef heifers, cobalt, digestibility

Introduction

Economic losses can be sustained from newly weaned cattle entering the feedlot if health is compromised. Cattle subjected to transportation and/or possible comingling are more susceptible to sickness (Duff and Galyean, 2007). Blecha et al. (1984) found that transportation stress causes suppressed immune function. Methods to alleviate stress may reduce morbidity and mortality.

Ruminant performance is related to fermentation of feed ingredients via the ruminal microbiome (Schwartz and Gilchrist, 1974). Emptying of ruminal contents is positively correlated with dry matter intake (Ulyatt et al., 1986) and can be affected by digestion, passage rate, or in some instances both (Balch and Campling, 1962; Troelson & Bigsby, 1964). As a result, increasing rate of digestion can increase animal intake and concurrently animal performance (Mertens, 1994).

Increased vitamin B_{12} synthesis by ruminal microbes may reduce stress. Cobalt is used to synthesize B12 and Smith (1987) showed that the conversion of dietary Co to vitamin B_{12} is generally very low; therefore supplemental Co may be needed. In addition to synthesis supplemental Co may also impact the rumen environment and increase fiber digestion and energy production. Cobalt can be supplemented using inorganic or organic sources. Several studies have evaluated multiple organic mineral mixes but have not looked at Co glucoheptonate supplementation in relation to an inorganic source. Therefore, our hypothesis is that Co supplementation will increase total tract apparent fiber digestibility, energy production and vitamin B_{12} production. The purpose of this study is to investigate the effects of Co supplementation on rumen fermentation characteristics and blood metabolites in stressed calves.

Materials and Methods

All animal handling and procedures were approved by the Institutional Animal Care and Use Committee of New Mexico State University.

Animals and Diet. Thirty Brangus and Brahman heifers (average BW = 256 ± 28.8 kg) were stratified by breed and used in a completely randomized design. Research was carried out at New Mexico State University (**NMSU**) campus animal research facilities in Las Cruces, New Mexico and Clayton Livestock Research Center (**CLRC**) in Clayton, New Mexico. In order to replicate

cattle management practices in the West a triphasic experiment was used to simulate a back grounding phase (**BP**) after weaning, transportation stress phase (**SP**), and a post stress phase (**PSP**). All heifers were fed a forage basal diet (Table 1) for all phases of the experiment consisting of alfalfa, Sudan hay, and Bermuda grass. The basal diet was formulated to meet or exceed (NRC, 1996) requirements for growing heifers and supplied 13.3% CP and 55.4% NDF, and 0.35 mg/kg of Co (DM basis). Supplemental treatments included: 1) 0 mg Co heifer^{-1}•d^{-1} (**CON**), 2) 12.5 mg Co from CoSO4 heifer^{-1}•d^{-1} (**SO4**) and 3) 12.5 mg Co from cobalt glucoheptonate heifer^{-1}•d^{-1} (**COPRO**). All treatments were pelleted using fine ground corn as a carrier. Treatments were fed prior to feeding the basal diet over the course of the study at a rate of 134.6 g hd^{-1}d^{-1}, 110.5 g hd^{-1}d^{-1}, and 128.1 g hd^{-1}d^{-1} for CON, SO4, and COPRO, respectively. The basal diet was not fed until supplements were completely consumed.

Adaptation of heifers to basal diet and treatments occurred in BP (27 d) where heifers were randomized to treatment and pens in groups of 5. Heifers were allowed *ad libitum* access to the basal diet and were allotted 137.5 m^2 of pen space and over 1 m bunk space hd^{-1}. Pens were dirt floored and equipped with automatic water fountains. Following completion of BP, SP began with the transportation of all heifers NMSU to CLRC (795 km). Upon arrival at CLRC, heifers were overnighted in a single holding pen with *ad libitum* access to water where cattle returned to NMSU the following day. Upon completion of SP, heifers remained on assigned treatments with *ad libitum* access to the basal diet but were randomized to individual pens for PSP (15 d). Individual water buckets and feeders were used in each pen with approximately 44.6 m^2 of pen space.

Sampling and Analysis. Feed refusals were collected, weighed, and recorded daily. Grab samples (approximately 20 g) from the orts were frozen at -20° C until further analysis. Approximately 40 mL of ruminal fluid was collected via oral lavage (Lodge-Ivey et al., 2009) prior to transportation (**SPBASE**), at arrival to CLRC (**SPARR**), on the following morning after returning to NMSU (**SPFINAL**), and on d 14 of SPS. pH was measured on all ruminal fluid samples using a portable pH probe (Accumet AP72, Fisher Scientific, Waltham, MA) within 5 min of collection. Ruminal samples were aliquotted for analysis, placed on ice, and subsequently frozen at -20° C. Ruminal fluid was acidified upon collection with 2 mL of 5% HCl for ammonia quantification. Ruminal ammonia was determined using the protocol by Broderick and Kang (1980) adapted to a microtiter plate (BioTek Instruments Inc., Winooski, VT) at a wavelength of 630 nm. Ruminal VFA profiles were measured using a gas chromatograph (Agilent Technologies 7890 A, Santa Clara, CA) according to the method of Goestch and Gaylean (1983). Blood were collected at the same time as ruminal fluid samples with exception of SPFINAL, where blood was taken immediately upon arrival to NMSU and ruminal fluid was collected 16 h later due to cattle fasting for 48 h. All blood samples were immediately placed on ice until further processing. Blood tubes were centrifuged (Dupont, Sorvall RT 6000B) at 1200 x g for 20 min at 4° C. Serum was poured off and stored in plastic vials at -20° C before it was analyzed for Co, cortisol, NEFA, and haptoglobin. Analysis for Co in ruminal fluid and blood was carried out at by a commercial laboratory (SDK Laboratories, Hutchinson, KS). Serology analysis for cortisol, NEFA, and haptoglobin conducted by out at New Mexico Department of Agriculture Veterinary Diagnostic Services (Albuquerque, NM). In order to calculate digestibility of diet, fecal output was estimated by orally bolusing 5 g of titanium dioxide twice daily from d 5 to 14 of PSP. Fecal grab samples were collected twice daily from d 10 to 14 of PSP. Titanium from the feces was extracted according to Myers et al. (2004) and quantified using a microtiter plate reader (BioTek Instruments Inc., Winooski, VT) at a wavelength of 410 nm.

Statistical Analysis. The GLM procedure of SAS version 9.3 (SAS Inst. Inc., Cary, NC) was used to analyze ruminal fermentation characteristics, blood metabolites, and total tract digestibility by phase (i.e. SPBASE, SPARR, SPFINAL, and PSP). Animal was used as the experimental unit, treatments represented fixed effects and random error was accounted for in the error term. Means were calculated using LSMEANS. Treatment effect was considered significant when the probability of a greater *F* was $\leq$ 0.05 and a tendency when *F* was $\leq$ 0.10. When *F*-tests were significant, mean separations were performed using PDIFF. Single degree of freedom orthogonal contrasts were used to compare effects of Co (COPRO and SO4) vs. no Co and COPRO vs. SO4.

Results

By supplying ruminal microbes with optimal substrate it may be possible to increase vitamin B_{12} production, fiber digestibility and VFA production. Furthermore, increased intake and energy supply may help cattle better manage transportation, fasting, and receiving stress. The effects of treatment on rumen fermentation, and blood metabolites are shown by phase; SPBASE, SPARR, SPFINAL, and PSP. Data are shown in table 1.

During SPBASE, treatment did not affect ruminal pH ($P \geq 0.16$). Ruminal ammonia tended to be lower ($P = 0.07$) for Co supplemented heifers than CON. Total VFA production was lower ($P = 0.01$) for Co supplemented heifers than CON. Molar proportion of propionate was not different ($P \geq 0.41$). Ruminal Co concentration was highest ($P < 0.01$) for COPRO and lowest for CON. However, treatment or source of Co did not alter ($P \geq 0.29$) serum Co concentrations. Analysis of pre-shipping blood metabolites indicated a tendency for NEFA to be higher ($P = 0.08$; data not shown) in Co supplemented heifers than CON but did not differ ($P = 0.92$) between Co source. No further differences in NEFA levels were seen between treatments in the remaining phases (data not shown).

After transportation ruminal pH in SPARR tended to be greater ($P = 0.10$) in Co supplemented heifers. Ruminal acetate was higher ($P = 0.03$) for COPRO and CON than for SO4 during SPARR. Though the acetate:propionate ratio was also higher for COPRO than SO4 ($P = 0.01$) at SPARR, total VFA, propionate, and butyrate proportions did not differ ($P \geq 0.51$). A tendency

for Haptoglobin to be higher ($P = 0.10$) in Co supplemented cattle was seen at arrival to CLRC. The only differences seen for SPFINAL was a tendency for ruminal Co concentration in Co supplemented heifers to be higher ($P = 0.06$) than CON.

During PSP, pH tended to be lower ($P = 0.08$) for Co supplemented heifers than CON. Ruminal ammonia N was higher ($P = 0.03$) for Co supplemented heifers during PSP. Total VFA production was higher in Co supplemented ($P = 0.01$) than CON. Cortisol and Haptoglobin were higher ($P = 0.02$, $P = 0.05$, respectively) in CON than Co supplemented heifers. During the PSP, no differences ($P \geq 0.85$) were found in total tract apparent organic matter or neutral detergent fiber digestibility.

Discussion

Optimal pH for fiber digesting bacteria is 6.9 to 7.0 (Weimer, 1996). Ruminal pH is largely affected by the fermentation of OM, production of fermentation acids (Mouriño, et al., 2001). Cellulolytic bacteria are sensitive to pH below 6.0 (Stewart, 1977) but all pH values throughout the study were 6.8 or higher indicating that pH did not likely affect fiber fermentation.

A converse response was seen for ruminal ammonia N from SPBASE to PSP. At SPBASE, no Co supplementation tended to increase ammonia N production, but after the stress phase it increased ammonia N at PSP. Throughout this experiment ruminal ammonia N levels were adequate to sustain normal rumen function and production of microbial crude protein (Satter and Slyter, 1974).

In vitro effects of Co supplementation on total VFA production are difficult to interpret and to our knowledge *in vivo* effects have not been reported. In the current study, the CON diet contained typical levels (0.35 mg Co kg^{-1} DM) of Co for a forage based diet while the supplemented treatment groups greatly exceeded (2.56 mg Co kg^{-1} DM) NRC Co requirements. The increase in PSP total VFA production with Co supplementation did agree with *in vitro* results from Tiffany et al., (2006) where total VFA production was increased ($P = 0.05$) with Co levels from 0.10 mg Co kg^{-1} to 1.0 mg Co kg^{-1} (DMB), albeit different levels were used between studies.

After transportation at SPARR acetate proportion was higher for CON and COPRO than SO4. Zhang et al., (2013) found that under conditions of short term feed restriction, absorption of SCFA is reduced largely due to a decreased acetate absorption rate. The mechanism for decreased acetate absorption is still unknown, but would result in higher ruminal proportions of acetate.

Lower Co concentrations in the rumen for the SO4 treatment may indicate a faster passage rate out of the rumen with the liquid fraction. This may additionally create an environment for higher vitamin B_{12} production with Co glucoheptonate supplementation. Research by Brown and Zeringue (1994) however indicated an increased solubility for complexed, chelated, and proteinated trace minerals when compared to inorganic sources. Although animals were not supplemented prior to sample collection during SPFINAL, heifers previously supplemented with Co retained elevated levels of ruminal Co. Intake was not measured during this phase, but it may be possible that Co supplemented heifers consumed more basal diet than CON at SPFINAL. This is of importance because one of the most significant impacts of stress is reduced feed intake (Loerch and Fluharty, 1999).

Tiffany et al., (2003 and 2006) demonstrated an increase in serum vitamin B_{12} when Co supplementation was increased from 0.10 mg/kg to 1.0 mg/kg (DMB). Vitamin B_{12} absorption is regulated and Co is not used in any other known processes in the bovine. Haptocorrin and intrinsic factor (Kozyraki and Cases, 2012), two binding proteins, must first bind to vitamin B_{12} which then allow attachment to cubulin receptors and ultimately phagocytosis (Kapadia et al., 1985). However, divalent metal ion transporters do exist in cattle (Hansen, 2008) with low divalent metal ion specificity (Gunshin, 1997). Enterocytic absorption of Co may occur, suggesting serum Co concentrations may be an inaccurate predictor of serum vitamin B_{12}. Serum vitamin B_{12} levels may not accurately predict actual vitamin B_{12} levels either as demonstrated by Price et al., (1993) who found that significant quantities of vitamin B_{12} remain undetected due to binding of vitamin B_{12} to transcobalamin I (Brada et al., 2000).

Many blood metabolites can be indicators of a humoral response to stress. Those of interest to this study were cortisol, NEFA, and haptoglobin. A slight tendency for lower NEFA level at early transportation may indicate that heifers supplemented with Co see an initial stress response earlier than CON. This was however abated the following day in SPFINAL where cattle were transported back to NMSU. Basal NEFA levels may indicate a higher metabolic rate for non-stressed heifers. Cortisol levels were not affected by Co supplementation until PSP. Interestingly, cortisol levels were numerically higher at SPBASE than at SPFINAL and PSP. Similar results (Blecha et al., 1984) in cortisol levels were seen when cattle were shipped 700 km after a back grounding period but did not increase cortisol levels. Low levels of haptoglobin in CON at SPARR may indicate increased hemolysis due to transportation stress. This was reversed in PSP where CON had higher haptoglobin levels. Circulating levels of haptoglobin may have remained elevated after compensating for higher serum hemoglobin. No hematologic levels were measured.

While no difference was seen in total tract apparent digestibility, the site of digestion may have been altered. Increasing ruminal fiber digestibility would be of interest as ruminants can better utilize ruminal microbial fermentation products than that of colonic fermentation.

Implications

Providing supplemental cobalt to stressed calves mediated stress responses and increased total volatile fatty acid production. Therefore, cobalt supplementation may allow stressed calves to more effectively deal with stresses and recover sooner. Cobalt source does not appear to be a factor. Further research is needed to determine the efficiency microbial vitamin B_{12} production including pathways of the various vitamin B_{12} analogues in stressed

calves. Also, the relationship between ruminal vitamin B_{12} synthesis and the capacity for host absorption needs further delineation.

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Table 1. The effect of cobalt supplementation on digestibility, ruminal and blood metabolites when calves were stressed due to transportation.

	Treatments[1]					Contrasts[2]	
Item	CON	COPRO	SO4	SEM	*P*-value	CON vs. Cobalt	COPRO vs. SO4
SPBASE[3]							
pH	7.0	7.2	7.2	0.08	0.32	0.13	0.96
Ammonia N, mM	3.1	2.3	2.6	0.31	0.13	0.07	0.42
Total VFA mmol	69.1[a]	56.8[b]	52.1[b]	4.24	0.02	0.01	0.44
Acetate, mol/100mol	74.3	74.0	74.5	0.38	0.91	0.80	0.71
Propionate, mol/100mol	14.6	14.6	14.3	0.31	0.63	0.58	0.43
Acetate:propionate	5.1	5.1	5.3	0.13	0.59	0.56	0.39
Ruminal Cobalt, ppm	0.02	0.11	0.06	0.01	< 0.01	< 0.01	< 0.01
Serum Cobalt, ppm	0.07	0.15	0.09	0.04	0.32	0.29	0.28
Serum Cortisol, pmol/dL	2.1	2.5	1.9	0.41	0.60	0.98	0.32
Haptoglobin	9.7	18.2	17.9	5.96	0.53	0.26	0.97
SPARR[3]							
pH	7.4	7.6	7.5	0.08	0.16	0.10	0.33
Ammonia N, mM	3.6	3.3	2.9	0.47	0.62	0.40	0.62
Total VFA mmol	31.3	26.3	31.9	3.69	0.51	0.64	0.29
Acetate, mol/100mol	65.9[a]	73.4[a]	44.3[b]	7.71	0.03	0.46	0.01
Propionate, mol/100mol	14.6	14.1	14.5	0.36	0.51	0.44	0.39
Acetate:propionate	4.5[a]	5.3[ab]	3.1[ac]	0.55	0.03	0.69	0.01
Ruminal Cobalt, ppm	0.01	0.02	0.02	0.01	0.59	0.33	0.75
Serum Cobalt, ppm	0.03	0.02	0.05	0.01	0.29	0.82	0.12
Serum Cortisol, pmol/dL	2.9	2.0	2.1	0.51	0.39	0.17	0.87
Haptoglobin	10.1	31.9	33.7	10.82	0.25	0.10	0.91
SPFINAL[3]							
pH	6.9	6.9	6.8	0.12	0.47	0.51	0.30
Ammonia N, mM	1.6	1.6	2.2	0.35	0.38	0.58	0.20
Total VFA mmol	64.6	62.7	71.4	4.09	0.30	0.63	0.14
Acetate, mol/100mol	75.9	77.2	76.4	0.60	0.34	0.24	0.38
propionate, mol/100mol	15.0	14.9	15.0	0.33	0.52	0.58	0.32
Acetate:propionate	5.0	5.2	5.0	0.14	0.40	0.43	0.28
Ruminal Cobalt, ppm	0.01	0.04	0.02	0.01	0.08	0.06	0.23
Serum Cobalt, ppm	0.02	0.02	0.04	0.01	0.35	0.71	0.17
Serum Cortisol, pmol/dL	1.7	1.5	1.1	0.31	0.30	0.22	0.38
Haptoglobin	13.6	43.5	43.1	18.9	0.42	0.19	0.99
PSP[3]							
pH	7.2	7.0	7.1	0.06	0.19	0.08	0.69
Ammonia N, mM	3.3	4.6	4.9	0.52	0.08	0.03	0.70
Total VFA, mmol	63.2[a]	79.1[b]	72.7[c]	3.06	<0.01	<0.01	0.15
Acetate, mmol/100 mol	74.1	74.3	74.0	0.32	0.76	0.96	0.47
Propionate, mmol/100 mol	14.7	14.9	15.0	0.21	0.41	0.21	0.69
Acetate:Propionate	5.1	5.0	4.9	0.08	0.48	0.27	0.60
Apparent total tract OM digestibility, %	79.3	79.3	79.1	1.15	0.99	0.95	0.58
Apparent total tract NDF digestibility, %	78.4	78.9	78.1	1.00	0.85	0.95	0.90
Ruminal cobalt, ppm	0.06	0.12	0.10	0.02	0.10	0.04	0.47
Serum cobalt, ppm	0.08	0.06	0.08	0.02	0.76	0.64	0.57
Cortisol	1.8	1.2	1.2	0.21	0.06	0.02	0.84
Haptoglobin, mg/dl	55.3	27.5	10.8	14.47	0.11	0.05	0.42

[1]Treatments: no supplemental Co (CON), 12.5 mg heifer^{-1}•d^{-1} of Co glucoheptonate (COPRO), and 12.5 mg heifer^{-1}•d^{-1} of cobalt sulfate (SO4).

[2] Single degree of freedom orthogonal contrasts: Co (COPRO and SO4) vs. no Co and COPRO vs. SO4.

[3] SPBASE = prior to transportation stress, SPARR = after transportation of 795 km, SPFINAL = after approximately 12 h without feed but ad libitum access to water and transportation of 795 km, and PSP = 15 day individual feeding period after stress induction

[a,b,c]Means within a row with unlike superscripts differ ($P < 0.05$).

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REGULATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) BY (C-X-C MOTIF) LIGAND 12 (CXCL12) IN OVINE CARUNCLE EXPLANTS

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ABSTRACT: Vascular endothelial growth factor (**VEGF**) is a potent inducer of vascularization, and plays important roles in vascularization of the placenta, benefiting overall fetal growth and development. For this reason, it is imperative to determine signaling mechanisms that induce VEGF synthesis during early gestation in ruminants. We previously demonstrated increased mRNA expression for C-X-C chemokine receptor four (**CXCR4**) and its ligand, (C-X-C motif) ligand 12 (**CXCL12**) in caruncle tissue on d 20 of pregnancy compared to non pregnant ewes and d 25 and 30 of pregnancy in fetal extraembryonic membrane tissue. Further, expression of CXCL12 and CXCR4 paralleled the pattern of VEGF expression. Therefore, the objective of the current study was to determine if caruncle tissue treated with CXCL12 affects VEGF expression using an in vitro model. Caruncle tissue was collected from mixed aged western whiteface ewes (n=3) on d 13 of the estrous cycle and tissue culture explants were used for the study. Explants were treated with either 0.1% BSA, which served as vehicle control (**VEH**) or increasing concentrations of recombinant CXCL12 (25, 50, 250 or 500 ng/mL) for 12 h. Real time PCR and Western blot analysis was used to assess relative mRNA levels and protein expression respectively for VEGF. Relative mRNA and protein expression levels were normalized by standard methods, and subjected to ANOVA with Newman-Keuls *post hoc* test to determine significant differences. The mRNA levels for VEGF did not change with treatments. However, VEGF protein was higher ($P < 0.05$) in VEH compared to explants treated with CXCL12 at 25, 50, and 250 ng/ml. Explants treated with 500 ng/ml of CXCL12 displayed a tendency (P=0.06) for decreased VEGF protein compared to VEH. Because VEGF protein in caruncle tissue decreased following a dose response treatment with CXCL12, CXCL12 may promote VEGF secretion, thereby functioning in an autocrine or paracrine fashion and stimulating angiogenesis needed for proper placental development. The relationship between CXCL12 and VEGF may lead to new insights into novel therapeutic targets for placental vascularization, thus aiding fetal growth and survival.

Key words: caruncle, CXCL12, sheep, VEGF

INTRODUCTION

The placenta plays a vital role in metabolic exchange of nutrients, respiratory gases, and waste between the fetal and maternal systems. Increased placental blood flow is critical for fetal growth and development (Reynolds et al., 2013). Vascular endothelial growth factor (**VEGF**) is a potent inducer of angiogenesis and aids in placental vascular growth and remodeling (Carmeliet et al., 1996; Ferrara et al., 2003). In VEGF gene knockout (**KO**) mice, embryonic lethality occurs by d 11 of pregnancy, with prominent cardiovascular defects, and placenta abnormalities (Carmeliet et al., 1996; Ferrara et al., 1996). Increased mRNA expression for VEGF and its receptors occurs in the maternal placenta of sheep during early pregnancy (Grazul-Bilska et al., 2010), with similar reports noted in cattle (Pfarrer et al., 2006). In addition to VEGF, (C-X-C motif) ligand 12 (**CXCL12**) also instigates angiogenesis and KO mice for CXCL12, or its receptor, C-X-C chemokine receptor four (**CXCR4**) results in serious vascular abnormalities, which are not observed in any other chemokine or chemokine receptor KO mice (Tachibana et al., 1998; Nagasawa, 2001). Interestingly, in endothelial cells, VEGF enhances CXCL12 and CXCR4 production, establishing a positive-feedback loop in which VEGF induces CXCR4 and CXCL12 expression, and conversely CXCL12/CXCR4 interactions enhance VEGF expression by these cells consequently linking classic angiogenic factors to chemokine-induced angiogenesis (Rosenkilde and Schwartz, 2004). Previously, we observed an increase of mRNA for VEGF, its two receptors and CXCL12, and CXCR4 on d 25 and/or 30 of pregnancy compared to d 20 in ovine fetal extraembryonic membrane tissues (Quinn et al., 2012). Our current objective was to determine if caruncle tissue treated with CXCL12 affects VEGF expression using an in vitro model. We hypothesized that CXCL12 would promote VEGF secretion from caruncle tissue potentially eliciting angiogenesis in an autocrine or paracrine fashion.

MATERIALS AND METHODS

All experimental procedures using animals were reviewed and approved by New Mexico State University Animal Care and Use Committee.

Animals and Tissue Collection. Ewes (n = 3) were anesthetized with sodium pentobarbitol on d 13 of the

estrous cycle. The reproductive tract was removed using a mid-ventral laparotomy and caruncle tissue was collected and directly placed in a 50 mL conical with media (Dulbecco's Modified Eagle Medium (DMEM) F12 supplemented with 10% fetal bovine serum (FBS) and 100 IU penicillin and 100 g/mL streptomycin) for transfer to cell culture. Ewes were euthanized by exsanguination.

Cell Culture. For each ewe, 0.2 g of caruncle tissue was minced into small explants and transferred to 6-well plates containing complete medium (DMEM F12 supplemented with 10% FBS and penicillin/streptomycin). Explants (n = 3 per treatment) were treated with either 0.1% bovine serum albumin (BSA), which served as vehicle control (VEH) or increasing concentrations (25, 50, 250, or 500 ng/mL) of recombinant CXCL12 (Pepro Tech.Rocky Hill, NJ). Explants were incubated at 37°C with slow agitation. After 12 h of treatment, media was aspirated, and each explant was washed with 2 mL of phosphate buffered saline (PBS). Explants were collected and snap frozen in liquid nitrogen, and stored at -80°C for subsequent RNA and protein isolation.

RNA and Protein Isolation. Total RNA was extracted from caruncle explants using Tri Reagent (Molecular Research Center Inc., Cincinnati, OH) per the manufacturer's directions. RNA was eluted in nuclease-free water and subsequently treated with DNase using the TURBO DNA-free kit (Ambion, Foster City, CA) to ensure samples were not contaminated with genomic DNA. The quantity and purity of RNA was determined using a Nanodrop-2000 spectrophotometer (Thermo Scientific, Waltham, MA). RNA samples were stored at -80°C until further analysis. Protein was isolated from caruncle explants by homogenizing 100 mg of tissue in 1 mL of Radio-Immunoprecipitation Assay (RIPA) buffer (50 mM Tris (pH 7.4), 2 mM EDTA, 150 mM NaCl, 0.1% sodium dodecyl sulphate, 1.0% TritonX-100) supplemented with phosphatase and protease inhibitor cocktail tablets (Roche Applied Science, Germany). Samples were placed on ice for 15 min and then centrifuged at 12,000 × g for 10 min at 4°C and supernatants subsequently removed and stored at -80°C. Concentrations of protein were determined using BCA protein assay (Pierce, Rockford, IL).

Real-time polymerase chain reaction (qPCR). Analysis of mRNA expression was completed using qPCR as previously published (Ashley et al., 2011). Briefly, cDNA was synthesized and qPCR was performed using a CFX96 Touch^{tm} Real-Time PCR Detection System (BioRad, Hercules, CA). The specific primers employed included: GAPDH forward primer, 5'-TGACCCCTTCATTGACCTTC-3', GAPDH reverse primer, 5'-CGTTCTCTGCCTTGACTGTG-3', and VEGF forward primer, 5'-TCACCAAAGCCAGCACATAG-3', VEGF reverse primer, 5'-AAATGCTTTCTCCGCTCTGA-3'. The GAPDH amplicon did not change across treatments and was used to normalize each target mRNA by using the ΔCq (target Cq - GAPDH Cq) values (Schmittgen and Livak, 2008). Data are represented by graphing $2^{-\Delta\Delta Cq}$ values. For each mRNA target, the amplicon was sequenced to ensure that each gene of interest was correctly amplified. Amplification efficiencies were determined using a 10-fold dilution series of cDNA for each primer set and each amplified at 95 to 110% efficiency.

Western Blot Analysis. Protein lysates were collected from ovine caruncle explants as described above. Equal concentrations of protein (50 µg per well) were separated by SDS-PAGE using 10% polyacrylamide gels followed by transfer to methanol activated polyvinyl diflouride (PVDF) membranes for immunoblotting. After blocking in 5% non-fat milk made in Tris-buffered saline plus tween (TBST) (68.4 mM Tris Base, 10 mM NaCl, 0.10% tween-20, pH 7.6) for 1 h at room temperature, membranes were incubated with VEGF (sc-507) primary antibody (Santa Cruz Biotechnology, Inc. Santa Cruz, CA) at 1:1,000 dilution in 5% non-fat milk made in TBST. A secondary donkey anti-rabbit IgG-horseradish peroxidase antibody (sc-2317) at a dilution of 1:5,000 was used. Proteins were visualized by SuperSignal® West Dura Extended Duration Substrate kit (Thermo Scientific, Rockford,IL) and detected using the ChemiDoc ™ XRS and Image Lab Software Version 3 (BioRad Laboratories, Hercules, CA). Beta tubulin protein was also determined to further demonstrate equal loading of protein. Anti-Beta tubulin antibody (sc-55529) was used at a 1:1,000 dilution and an anti-mouse (sc-2005) secondary antibody at a dilution of 1:5,000 was used.

Statistical Analysis. The qPCR data were subjected to ANOVA analysis appropriate for a completely randomized design. When a significant treatment effect ($P < 0.05$) was detected, means were separated using Newman-Keuls test on normalized Cq values using Prism (Version 5 from GraphPad Software, Inc.). The chemiluminescent signals for Western blots were quantified using the mean value (intensity) with the Image Lab software program for each band of interest and were divided by the mean value (intensity) for Beta tubulin. Significant changes were determined at $P < 0.05$ using an unpaired, two-tailed student's t-test on Prism (Version 5 from GraphPad Software, Inc.).

RESULTS AND DISCUSSION

In caruncle explants, VEGF mRNA did not change throughout CXCL12 treatments (Fig. 1). However, VEGF protein was greater ($P < 0.05$) in VEH compared to explants treated with CXCL12 at 25, 50, and 250 ng/mL. Explants treated with 500 ng/mL of CXCL12 displayed a tendency ($P = 0.06$) for decreased VEGF protein compared to VEH (Fig. 2). Due to a decrease in VEGF protein following dose response treatment with CXCL12, CXCL12 may be promoting VEGF secretion in a paracrine or autocrine fashion. Thus, the decrease in VEGF protein observed in the explants may be due to the explants actually secreting VEGF. In human umbilical vein endothelial cells, VEGF enhances the expression of CXCR4, and subsequently, CXCL12 can further enhance VEGF expression (Salcedo et al., 1999). In bovine ovarian antral follicles, CXCL12 and VEGF gene expression increased similarly, following antral follicle development (small-large), providing a role for both CXCL12 and VEGF in ovarian angiogenesis (Skinner et al., 2008). In ruminants, most placental growth occurs during early gestation, with

limited growth taking place during the last half (Reynolds and Redmer, 1995). In mammals approximately 30% of embryonic losses occur during early gestation, with an even greater percentage (50%) seen in humans (Edey, 1969). Studying placental development during early gestation is critical for understanding the development and growth of the fetus throughout pregnancy. Previous data from our lab identified an increase of CXCL12 and VEGF mRNA and/or protein on d 25 and 30 compared to d 20 of pregnancy. Receptors for CXCL12 and VEGF followed a similar pattern, with CXCR4 and VEGFR1 increasing on d 25 and 30 compared to d 20, and VEGFR2 peaking specifically on d 25 compared to d 20 and 30 (Quinn et al., 2012). Data from the current study support our previous findings and further identifies how CXCL12 may promote VEGF. It is well understood that transplacental exchange is vital for fetal health, and relies heavily on angiogenesis and formation of new vascular beds. Vascular Endothelial Growth Factor is a potent inducer of vascularization and its importance during placental development has been well identified; however, there is still a lack of information on upstream signaling events that regulate VEGF expression and secretion. Identifying new signal mechanisms promoting VEGF synthesis and secretion could provide new insights into other proteins involved in promoting placental development and fetal survival in livestock.

IMPLICATIONS

Due to the decrease in VEGF observed in caruncle explants after treatment with CXCL12, CXCL12 may promote secretion of VEGF from the tissues. Few studies have addressed the association between CXCL12 and VEGF during pregnancy in ruminants and how this system is potentially driving growth and vascularization of the placenta. Using an in vitro model, we have identified a potential synergistic relationship between CXCL12 and VEGF. Further establishing this association in ruminants may be beneficial for livestock producers, as it could lead to novel therapeutics targeting placental vascularization, thus aiding in fetal survival and growth throughout pregnancy.

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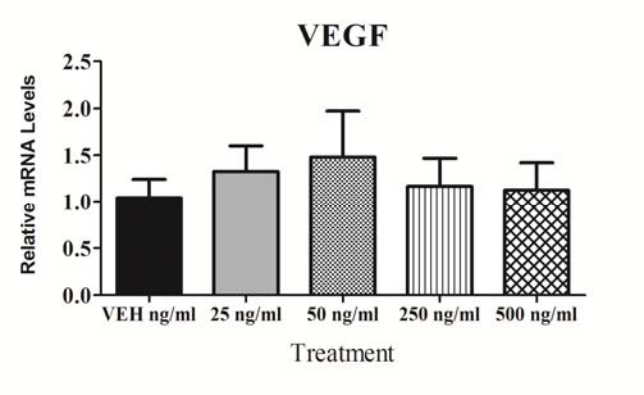

Figure 1. Expression of mRNA for VEGF remained constant across treatments tested. Increasing concentrations (25, 50, 250 and 500 ng/mL) of recombinant CXCL12 or a control (VEH) were administered to caruncle tissue explants (n = 3 per treatment). Relative mRNA levels for VEGF were measured using qPCR. No differences were seen in VEGF mRNA expression across treatments.

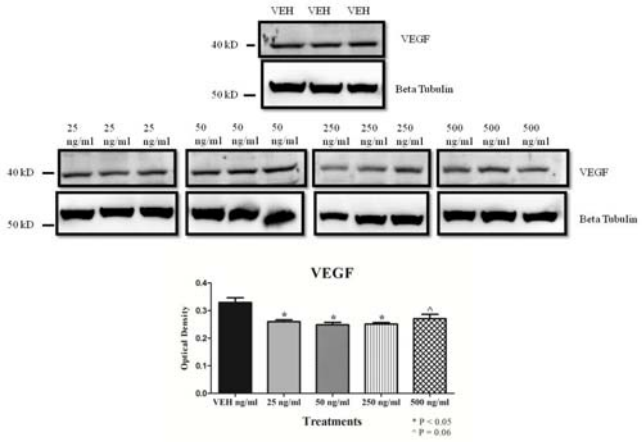

Figure 2. Protein for VEGF decreased in caruncle explants treated with recombinant CXCL12. Equal concentrations of caruncle protein were subjected to SDS –PAGE and Western Blot Analysis was performed to verify VEGF protein. Data represents 3 ewes per treatment. To further verify equal loading, the same protein samples were immunodetected for beta tubulin. Molecular weight is also indicated for each protein of interest. VEGF protein was greater ($P < 0.05$) in VEH (control) compared to explants treated with CXCL12 at 25, 50, and 250 ng/ml (represented by asterisk). Explants treated with 500 ng/ml of CXCL12 displayed a tendency ($P = 0.06$) for decreased VEGF protein compared to VEH.

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EFFECT OF PUBERTAL STATUS AND NUMBER OF ESTROUS CYCLES PRIOR TO THE BREEDING SEASON ON PREGNANCY RATE IN BEEF HEIFERS

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ABSTRACT: Three experiments were conducted to evaluate whether pubertal status prior to breeding influences pregnancy rate in beef heifers. Records were collected at West Central Research and Extension Center, North Platte, NE from 2002 to 2011 (Exp. 1; n = 1,005) and Gudmundsen Sandhills Laboratory, Whitman, NE from 1997 to 2011 (Exp. 2; n = 1,253). Heifers in Exp. 1 and 2 were classified as either being pubertal or non-pubertal at the start of breeding. In Exp. 3, (n = 156) heifers were classified by number of estrous cycles (0, 1, 2, 3, or ≥ 4) exhibited prior to breeding. In Exp. 1 and 2, pubertal heifers were heavier ($P \leq 0.04$) and older ($P < 0.07$) at start of breeding and had greater ($P < 0.01$) overall pregnancy rate (94 vs. 88 ± 2%; 90 vs. 82 ± 2% in Exp. 1 and 2, respectively) than non-pubertal heifers. Pubertal heifers also tended ($P = 0.08$) to have greater AI pregnancy rate (62 vs. 56 ± 4%, Exp. 1), produced more calves within the first 21 d of calving ($P < 0.01$), and weaned older ($P = 0.05$), heavier ($P < 0.01$) calves than heifers that had not reached puberty (Exp. 2). In Exp. 3, heifers pubertal prior to the breeding season had greater (85 ± 8%, $P = 0.05$) pregnancy rates (68 ± 8%) than non-pubertal heifers and pregnancy rate tended ($P = 0.15$) to be influenced by the number of estrous cycles (68, 81, 91, 93, and 82 ± 9% for 0, 1, 2, 3, or ≥ 4 estrous cycles, respectively). Second season pregnancy rate was greater for heifers reaching puberty prior to first breeding (97 vs. 80 ± 7%, $P < 0.01$) and was influenced ($P = 0.03$) by the number of estrous cycles, where heifers having ≥ 2 estrous cycles had greater pregnancy rate (80, 87, 100, 97, and 98 ± 8% for 0, 1, 2, 3, or ≥ 4 estrous cycles, respectively) than non-pubertal heifers. Pregnancy rate was greater for heifers achieving puberty prior to breeding, which was influenced by age and BW. However, earlier onset of puberty did not significantly improve first pregnancy rates.

Key words: beef heifers, puberty, reproduction

Introduction

Replacement heifer development can significantly impact the profitability of a beef cattle operation. It is imperative replacement females be developed economically to have their first calf at 2 yr of age. In order for this to occur, puberty must be attained by approximately 13 to 15 mo of age. Heifers that conceive early in the breeding season calve earlier and wean heavier calves, increasing longevity and productivity within the herd (Short and Bellows, 1971; Lesmeister et al., 1973; Funston et al., 2012a). Breeding heifers at 13 to 15 mo of age may be a disadvantage to later maturing heifers, as pregnancy rates have been correlated with the percentage of heifers that reach puberty before or early in the breeding season (Short and Bellows, 1971; Perry et al., 1991). Byerley et al. (1987) demonstrated heifers inseminated on pubertal estrus had a decreased pregnancy rate compared with heifers inseminated on their third estrus. However, heifers inseminated on pubertal estrus were inseminated at an earlier date than heifers inseminated on the third estrus. Therefore, heifers inseminated on the pubertal estrus were younger and weighed less at breeding.

Recent research has demonstrated heifers developed to a lower pre-breeding BW achieved acceptable pregnancy rates (Funston et al., 2012b) and in a recent review on heifer development and lifetime productivity (Endecott et al., 2013), it was hypothesized that genetic selection with the implementation of EPD for traits such as growth, milk, carcass characteristics, and scrotal circumference may have contributed to changes in beef heifer reproductive performance over time.

Therefore, the objectives of this study were to determine the effect of pubertal status and the number of estrous cycles prior to breeding on pregnancy rates in beef heifers.

Materials and Methods

All animal procedures and facilities were approved by the University of Nebraska Institutional Animal Care and Use Committee.

Data were collected from the West Central Research and Extension Center (**WCREC**), North Platte (2002 to 2011; n = 1,005, **Exp. 1**) and Gudmundsen Sandhills Laboratory (**GSL**), Whitman (1997 to 2011; n = 1,253, **Exp. 2**; n = 156, **Exp. 3**). Heifers at WCREC were Angus based and synchronized with a melengestrol acetate-$PGF_{2\alpha}$ protocol (Funston and Larson, 2011) prior to AI. Approximately 10 d following AI, heifers were exposed to fertile bulls at a bull to heifer ratio of 1:50 for 60 d. Conception to AI was determined 45 d after AI by transrectal ultrasonography and final pregnancy rate was determined via transrectal ultrasonography 45 d following removal of bulls.

Data from GSL were collected on a spring calving herd of composite Red Angus × Simmental females. Heifers were exposed to bulls for 45 d at a bull to heifer ratio of 1:25. A single injection of $PGF_{2\alpha}$ (Prostamate, Teva Animal Health Inc., St. Joseph, MO, or Lutalyse, Pfizer Animal Health, New York, NY) was administered i.m. to heifers 108 h after placement with bulls. Pregnancy determination was performed via transrectal

ultrasonography approximately 45 d after the breeding season.

Pubertal status was determined by evaluating progesterone concentration in 2 blood samples collected via coccygeal venipuncture 10 d apart prior to the breeding season for Exp. 1 and 2. The number of estrous cycles prior to the breeding season in Exp. 3 was determined via serial blood collection every 10 d beginning in early January of each year until the beginning of the breeding season (late May). Heifers in Exp. 3 were further classified as non-pubertal or pubertal (0 vs. ≥ 1 estrous cycle) and as having exhibited 1 estrous cycle or greater than or equal to 2 estrous cycles, excluding heifers that had not reached puberty (1 vs. ≥ 2) prior to breeding to evaluate effects on pregnancy rate. Blood samples were stored at 4°C for serum separation by centrifugation (2,500 × g for 20 min at 4°C) within 24 h. Serum samples were stored at −20°C for subsequent analysis. Serum progesterone concentrations were determined by direct solid-phase RIA (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) without extraction as described by Melvin et al. (1999). Intra- and interassay CV were 4.2 and 2.8%, respectively. Progesterone concentration > 1 ng/ mL was interpreted to indicate ovarian luteal activity.

Statistical analysis. Data were analyzed using PROC GLIMMIX of SAS 9.2 (SAS Inst., Inc., Cary, NC). Means were separated using LSD. Effects of pubertal status or number of estrous cycles were considered to be significant when $P \leq 0.05$, a tendency when $P \leq 0.10$, or a trend when $P \leq 0.15$.

Results and Discussion

Exp. 1

Date of birth, BW, pregnancy rate and first calf characteristics of heifers classified by pubertal status prior to breeding are presented in Table 1. Julian birth date was similar ($P = 0.12$) for heifers that were pubertal or non-pubertal. Pubertal heifers had greater ($P < 0.01$) BW compared with non-pubertal heifers from weaning through final pregnancy diagnosis. Weaning to final pregnancy diagnosis ADG was similar ($P = 0.62$; 0.54 vs. 0.53 ± 0.05 kg/d for non-pubertal and pubertal, respectively) for pubertal and non-pubertal heifers providing evidence that differences in post-weaning BW were likely due to greater pre-weaning ADG for heifers that reached puberty prior to breeding. These results are consistent with previous research indicating pre-weaning growth exerts a greater influence on puberty than post-weaning growth (Patterson et al., 1992; Roberts et al., 2009). This also agrees with previous research reporting ADG prior to breeding has minimal impact on pubertal status and pregnancy rates (Funston and Deutscher, 2004; Martin et al., 2007; Funston and Larson, 2011; Larson et al., 2011).

Heifers that were pubertal prior to breeding tended ($P = 0.08$) to have greater AI pregnancy rate (62 vs. 56 ± 4%) and greater ($P < 0.01$) overall pregnancy rate (94 vs. 88 ± 2%) compared with non-pubertal heifers. Days to calving was decreased ($P < 0.01$) for pubertal vs. non-pubertal heifers, however, calf birth BW did not differ ($P = 0.92$; 34 ± .7 kg).

Exp. 2

Date of birth, BW, ADG, pregnancy rate and first calf characteristics of heifers classified by pubertal status prior to breeding are presented in Table 2. Heifers that were pubertal prior to breeding were born approximately 4 d earlier ($P < 0.01$) than non-pubertal heifers.

Heifer birth BW did not differ ($P = 0.28$) between groups. However, pubertal heifers had greater ($P < 0.01$) weaning and pre-breeding BW, and tended ($P = 0.08$) to be heavier at pregnancy diagnosis than non-pubertal heifers. Heifers that were pubertal prior to breeding had greater ($P < 0.01$) ADG from birth to weaning, similar to what is being hypothesized for Exp. 1. Heifers that did not reach puberty prior to breeding tended ($P = 0.09$) to have greater ADG from weaning to pre-breeding and had greater ($P < 0.01$) ADG from breeding to pregnancy diagnosis. The greater ADG from weaning to pregnancy diagnosis by non-pubertal heifers resulted in a similar ($P = 0.41$) BW at pre-calving.

Pregnancy rate was greater ($P < 0.01$) for pubertal heifers vs. non-pubertal heifers (90 vs. 84 ± 2%, respectively). A greater ($P < 0.01$) proportion of pubertal heifers calved within the first 21d of the calving season compared with heifers classified as non-pubertal prior to breeding. Date of calving was 5 d earlier for heifers that were pubertal prior to breeding and their calves were heavier ($P < 0.01$) at birth and were heavier and older ($P < 0.05$) at weaning than calves from heifers that were not pubertal prior to breeding. At weaning, there was no difference (P > 0.90) in BW (317 ± 8.4 kg) and BCS (5.1 ± 0.1) between first calf heifers classified as pubertal or non-pubertal before start of breeding as heifers. Second season pregnancy rate was also similar ($P = 0.65$) between groups.

Exp. 3

Date of birth, BW, ADG, pregnancy rate, and first calf characteristics are presented in Table 3 for heifers classified by number of estrous cycles prior to the breeding season. Heifers had similar ($P = 0.34$) birth BW regardless of number of estrous cycles prior to breeding. There was a trend ($P = 0.12$) for heifers that had 3 estrous cycles prior to the breeding season to be born earlier and a tendency ($P = 0.10$) to have greater weaning BW compared with heifers that exhibited estrus ≤ 2 and ≥ 4 times.

Heifers exhibiting ≥ 4 estrous cycles were younger ($P < 0.01$; 409, 394, 379, 324 ± 6.3 d, for 1, 2, 3, and ≥ 4 estrous cycle groups, respectively) and had reduced ($P < 0.01$) BW at puberty than heifers exhibiting estrus ≤ 3 times. Heifers that exhibited ≤ 3 estrous cycles had similar ($P \geq 0.92$) BW at puberty. Birth to weaning ADG was similar ($P = 0.27$), however, heifers that had ≥ 4 cycles had lower ($P < 0.01$) weaning to puberty ADG compared with heifers that exhibited estrus ≤ 3 times. Heifer BW was similar ($P \geq 0.16$) at pre-breeding and pregnancy diagnosis.

There was a trend ($P = 0.15$) for pregnancy rate to increase with the number of estrous cycles exhibited prior to breeding. Heifers that were pubertal prior to breeding had greater ($P = 0.05$; 85 vs. 68 ± 8%) pregnancy rate than non-pubertal heifers. Pregnancy rate did not differ for heifers having 1 estrous cycle compared with heifers having ≥ 2 estrous cycles prior to breeding ($P = 0.68$; 81 vs. 85 ± 9%

for 1 and ≥ 2, respectively). In contrast, Byerley et al. (1987) reported pregnancy rate was decreased 21 percentage points for heifers inseminated at pubertal estrus compared with third estrus. In the current study, heifers were placed with bulls or AI on a common date resulting in similar age at breeding, whereas date of insemination in Byerley et al. (1987) was earlier for heifers at pubertal estrus compared with heifers inseminated on third estrus, resulting in heifers inseminated on first estrus being approximately 50 d younger at breeding.

Pre-calving BW tended to be greater for heifers exhibiting ≥ 3 estrous cycles than those exhibiting estrus ≤ 2 times prior to breeding. The proportion of heifers that calved within the first 21 d, calf birth date, and calf birth BW did not differ ($P \geq 0.20$) among estrous cycle classifications prior to breeding.

Heifers that were pubertal prior to the first breeding season had a greater ($P < 0.01$) second season pregnancy rate than heifers that were non-pubertal prior to the first breeding season (97 vs. 80 ± 7%). Second season pregnancy rate was greater ($P = 0.03$) for heifers having ≥ 2 estrous cycle prior to the first breeding season than heifers having ≤ 1 estrous cycle, however heifers that had 0 or 1 estrous cycle had similar ($P = 0.81$) second season pregnancy rates (80, 87, 100, 97, and 98 ± 8% for 0, 1, 2, 3, and ≥ 4 estrous cycle groups, respectively). Heifers with ≥ 2 estrous cycles prior to the first breeding season also tended ($P = 0.08$) to have greater second season pregnancy rate compared with heifers that had 1 estrous cycle (98 vs. 88 ± 6% for ≥ 2 and 1 estrous cycles, respectively).

Implications

In most beef operations heifers are inseminated to calve at approximately 24 mo of age. This requires heifers to attain puberty and conceive by 15 mo of age. It has been previously suggested that heifers should reach puberty 1 to 3 mo prior to the breeding season to obtain greater fertility. Results from this study suggest if a heifer attains puberty prior to the breeding season, acceptable pregnancy rates can be achieved regardless of the number of estrous cycles experienced prior to breeding. However, additional research is needed to further substantiate the potential impacts pubertal status prior to the start of breeding may have on second season pregnancy rates.

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Table 1. Birth date, BW, pregnancy rate, and first calf characteristics of heifers classified by pubertal status prior to breeding. (Exp. 1)

	Pubertal	Non-Pubertal	SE	*P*-value
n	695	310		
Julian birth date[1], d	78.9	81.9	1.5	0.12
Weaning BW, kg	270	232	4.3	<0.01
AI BW, kg	357	348	12.1	<0.01
AI pregnancy rate, %	61.9	55.5	3.7	0.08
Overall pregnancy diagnosis BW, kg	423	416	8.3	<0.01
Overall pregnancy rate, %	94.2	87.7	1.9	<0.01
Days to calving[2], d	284	288	2.0	<0.01
Calve within first 21d[3], %	77.8	66.2	5.1	<0.01

[1]Birth date was known for only a subset of heifers (n = 360).
[2]Days from start of breeding season to calving.
[3]Calved within the first 21 d of the calving season, d 1 refers to the day the first calf is born.

Table 2. Birth date, BW, ADG, pregnancy rate, and first calf characteristics of heifers classified by pubertal status prior to breeding. (Exp. 2)

	Pubertal	Non-Pubertal	SE	*P*-value
n	752	491		
Julian birth date, d	83.9	87.8	4.8	<0.01
Born first 21d[1], %	63.8	49.7	5.9	<0.01
Birth BW, kg	34.8	35.2	0.64	0.28
Weaning BW, kg	209	202	3.0	<0.01
Birth to weaning ADG, kg	0.79	0.77	0.5	<0.01
Pre-breeding age, d	428	424	2.9	<0.01
Pre-breed BW, kg	302	294	4.4	<0.01
Pre-breed ADG, kg	0.45	0.46	0.03	0.09
Pregnancy diagnosis BW, kg	368	365	4.4	0.08
Breeding to pregnancy diagnosis ADG, kg	0.64	0.68	0.05	<0.01
Pregnancy rate, %	90.0	84.2	2.0	<0.01
Pre-calving BW, kg	423	421	7.1	0.41
Calve within first 21d[2], %	79.1	67.0	4.3	<0.01
Calf Julian birth date, d	75	80	4.9	<0.01
Calf birth BW, kg	33	32	0.5	<0.01
Calf weaning BW, kg	187	177	5.4	<0.01
Calf weaning age, d	181	177	3.8	0.05
Cow BW at weaning, kg	417	417	8.4	0.99
Cow BCS at weaning	5.1	5.1	0.1	0.91
Second pregnancy rate , %	89.8	91.2	3.1	0.65

[1]Born within the first 21 d of calving season, d 1 is the day the first calf is born.
[2]Calved within the first 21 d of the calving season, d 1 is the day the first calf is born.

Table 3. Birth date, BW, ADG, pregnancy rate, and first calf characteristics of heifers classified by number of estrous cycles prior to breeding. (Exp. 3)

	0	1	2	3	≥4	SE	*P*-value
n	25	16	22	27	66	156	
Julian birth date, d	85.3	85.9	85.8	78.2	84.0	3.1	0.12
Born first 21d[1], %	67.8	80.8	73.1	93.0	78.7	9.3	0.24
Birth BW, kg	35.7	33.8	34.2	36.3	35.3	1.3	0.34
Weaning BW, kg	222	224	230	238	229	7.7	0.10
Age at puberty, d	-	409[a]	394[a]	379[b]	324[c]	6.3	<0.01
Puberty BW, kg	-	316[a]	323[a]	315[a]	260[b]	13.7	<0.01
Wean to puberty ADG, kg/d	-	1.08[a]	1.09[a]	1.09[a]	0.61[b]	1.09	<0.01
Pre-breed BW, kg	376	383	392	406	385	17.4	0.16
Pregnancy diagnosis BW, kg	362	365	366	380	364	13.2	0.27
Pregnancy rate, %	68.0	81.3	90.9	92.6	81.8	9.4	0.15
Wean to pregnancy diagnosis ADG, kg	0.48	0.49	0.48	0.49	0.47	0.04	0.79
Puberty to pregnancy diagnosis ADG, kg	-	0.52[ab]	0.38[c]	0.49[b]	0.57[a]	0.34	<0.01
Pre-calving BW, kg	426	426	441	455	435	16.1	0.10
Calve within first 21d[2], %	65.3	83.6	87.6	82.7	75.1	14.2	0.47
Calf Julian birth date, d	72.5	66.9	63.4	67.8	68.8	4.5	0.20
Calf birth BW, kg	30.7	30.5	31.2	31.9	31.5	1.4	0.78
Second pre-breed BW, kg	376	383	392	406	385	17.5	0.16
Second pregnancy rate, %	79.5[b]	87.2[ab]	100.0[a]	97.0[a]	97.9[a]	8.0	0.03

[a-c]Means without a common superscript differ ($P \leq 0.05$).
[1]Born within the first 21 d of calving season, d 1 is the day the first calf is born.
[2]Calved within the first 21 d of the calving season, d 1 is the day the first calf is born.

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EFFECTS OF ALFALFA PARTICLE SIZE ON INTAKE AND DIGESTABILITY IN CROSSBRED LAMBS

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ABSTRACT: Forage particle size can affect voluntary intake, passage rate of feed through the rumen, and the available surface area for microbial degradation. Each of these factors could affect animal performance. To evaluate the effects of forage processing, 12 crossbred lambs (5 mo of age, average BW = 33 ± 3.9 kg) were used in a completely random design to determine *in vivo* DMI, OM intake, DM, NDF, and OM digestibility with total fecal collection in metabolism crates. Treatments consisted of alfalfa hay fed long stem (28.44% > 4.75 mm, mean particle size of remainder 3.49 mm) or chopped to pass a 2.54 cm screen (4.06% > 4.75 mm, mean particle size of remainder 1.70). A molasses:salt mixture was sprayed onto each lamb's feed with a backpack sprayer (11.36 L water + 340.19 mL molasses + 226.80 g NaCl) to control dust and reduce particle selection. Lambs were adapted for 14 d to treatments and metabolism crates. The amount of alfalfa fed and refused was recorded and the fecal output collected twice daily for the next 7 d. Mean DMI was 1.97 and 1.85, and OM intake 1.80 and 1.64 kg•lamb^{-1}•d^{-1} for chopped and long stem hay treatments respectively. Mean digestibility was 47.23%, 39.94%, and 49.39% for the chopped hay, and 46.56%, 36.70%, and 47.99% for the long stem hay treatment for DM, NDF, and OM digestibility's, respectively. All measures of intake and digestibility did not differ ($P > 0.20$) between treatments. Therefore, feeding lambs chopped compared to long-stem alfalfa hay did not increase intake or decrease measures of digestibility.

Key Words: alfalfa hay, digestibility, intake, lamb, particle size

Introduction

Physical breakdown is important to the digestion of forage, affecting intake, passage through the digestive tract, and surface area of the feed (Jaster and Murpy, 1983). However, a lack of consensus persists in the literature regarding the impacts of forage particle size on intake and digestibility in livestock (Al-Saiady et al. 2010; Maulfair et al. 2011).

The objective of this study was to investigate the effects of alfalfa hay, fed either long stem or chopped to pass a 2.54 cm screen, on intake and digestion by lambs. The null hypothesis was that measures of intake and digestion would not vary by long stem or chopped hay treatments.

Methods and Materials

Animal care and handling was approved by the Montana State University Agriculture Animal Care and Use Committee. Twelve 5 mo old crossbred lambs (average BW = 33 ± 3.9 kg) were randomly assigned to treatments (n = 6). Small square alfalfa hay bales from a single source were randomly sorted for use in the long stem (fed directly from the bale) or chopped (2.54 cm screen in Art's Way 5105 grinder) treatments. A molasses and salt mixture (11.36 L water, 340.19 ml molasses, and 226.80 g NaCl) was sprayed onto each lamb's feed with a backpack sprayer to control dust and reduce particle selection. Hay treatments are described in Table 1 and Fig. 1. Three cores were taken from each long stem bale (Penn State Forage Sampler) and 12 grab samples were taken from the chopped hay before the start of the trial. Samples were combined by treatment for analyses of nutrient composition and particle size. The experimental period was 21 d and included a 7 d adaption to the diet, followed by a 7 d adaption to the metabolism crates and fecal bags. The final 7 d amount of feed fed and refused was weighed, and fecal output was collected. Feed was provided between 0600 to 0800 and 1600 to 1800 at 110% of the previous day's intake to provide ad-libitum access to feed. Fecal bags were emptied twice daily.

At the end of the study each lamb's total fecal output was weighted, recorded, and grab samples taken from both the fecal output and orts of each lamb. All samples were stored for later analysis. Longstem and chopped hay particle size distribution was determined by dry sieving (Cole Parmer 8" 4.75-0.075 mm). Samples of feed, orts, and feces were dried overnight at 50°C, and then ground (WileyMill) to pass a 2 mm and then 1 mm screen**.** Crude protein (Leco FP 528), DM, and OM content of samples were determined by AOAC (2012). Neutral detergent fiber was measured according to Van Soest et al. (1991). In situ samples were ground to pass a 2 mm screen (WileyMill) and analyzed according to Ørskov (2000).

Digestibility was calculated for DM, NDF, ADF, and OM using the equation:

$$\textbf{Digestibility} = \frac{(\textbf{kg fed x K}) - (\textbf{kg refused x K}) - (\textbf{kg feces x K})}{(\textbf{kg fed x K}) - (\textbf{kg refused x K})}$$

Where K is the coefficient. Data was analyzed with the PROC MIXED package of SAS (SAS Inst., Inc., Cary, NC). The model included the effects of treatment. Lamb was the experimental unit. Significant was declared at $P \leq 0.05$.

Results

The chopped treatment had 4.06% of particles greater than the 4.75 mm screen, with the mean of the remainder 1.70 ± 0.18 mm. The long stem had 28.44 % greater than the 4.75 mm screen, and a remaining mean of 3.49 ± 0.13 mm (Fig. 1). Mean DMI and OM intake was 1.97 and 1.80 kg•lamb^{-1}•d^{-1} for the chopped hay treatment, and 1.85 and 1.64 kg•lamb^{-1}•d^{-1} long stem hay treatments. Mean OM, DM, and NDF digestibilities were 49.39%, 47.23%, and 39.94% for the chopped hay treatment, and 47.99%, 46.56%, and 36.70% for the long stem hay treatment, respectively. There was no difference ($P \geq 0.20$) in the mean treatment effects of long stem and chopped alfalfa hay for DMI, OM intake, and measures of digestibility (Table 2).

Discussion

Al-Saiaday et al. (2010) also found that DMI and DM digestibility did not differ between alfalfa hay particle sizes of 9.5 mm, 14 mm, and 17.8 cm (average particle size unprocessed hay) fed at 25% of the diet to male lambs. Conversely, Jaster and Murphey (1983) found a higher DMI in conjunction with lower DM and NDF digestibilities in 3 particle sizes of alfalfa hay (ranging from ground to pass a 10.2 cm screen to unchopped) in a forage only diet fed to Holstein heifers. Additionally, 4 different particle sizes (12.7 mm, 4.75 mm, 3.06 mm, and 1.0 mm) of loose hay fed to wethers at 2 levels of fixed intake (2.4 and 1.1 times maintenance requirement) decreased *in vivo* OM digestibility with decreased particle size at both levels of intake (Alwash and Thomas, 1974). Cows and sheep fed hay at three different particle sizes (long = 28.7 mm, medium = 9.2 mm, fine = 2.9 mm) increased OM and NDF digestibility (determined by TiO_2 markers) from long to medium chopped hay, but had the lowest digestibility when fed fine chopped hay (Tafaj et al., 2001). In dairy cattle nutrition, 1.18 mm is considered a standard critical size for feed to have physiologic effects (Maulfair et al., 2011). Effects on intake and digestibility seem more apparent with smaller particle sizes, possibly because of proximity to 1.18 mm.

Many of the studies investigating particle size of forages on measures of digestibility combine the forage with a concentrate (Maulfair et al., 2011; Al Saiady et al., 2012; Nasrollahi et al., 2012). Tafaj et al. (2001) and Nasrollahi et al. (2012) saw interactions between fiber and non-structural carbohydrate digestion. Since only alfalfa hay was fed in our study, these interactions between hay and concentrate could account for some of the differences in results between this study and others. In addition, increasing forage particle size is associated with longer time spent chewing (Nasrollahi et al., 2012) and more effective chewing (Delgado et al., 1997). Since there was no difference in digestibility or intake for this study, mastication may have been sufficient to overcome differences in feed particle sizes prior to microbial fermentation.

Implications

The results indicate chopping alfalfa hay to pass a 2.54 cm screen does not beneficially impact measures of intake or digestibility compared to feeding long stem hay.

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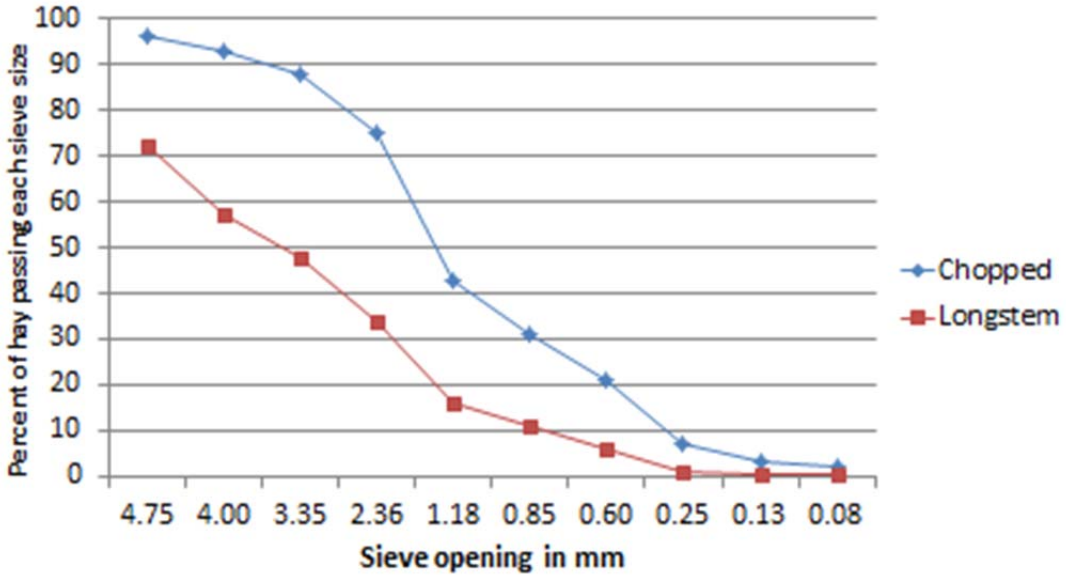

Figure 1. Particle size distribution of long stem and chopped alfalfa hay treatments determined by dry sieving (Cole Parmer 8" 4.75-0.075 mm).

Table 1 Nutritional analysis and *in situ* digestibility of longstem and chopped alfalfa hay treatments (DM basis).

	Treatment	
Item	Chopped hay[1]	Long stem hay[2]
OM, %	90.58	89.78
CP, %	13.53	13.96
NDF, %	63.64	62.56
ADF, %	51.50	47.79
In situ digestibility, %	55.30	43.20

[1]Chopped hay = square baled alfalfa hay ground to pass a 2.54 cm screen (Art's Way 5105).
[2] Long stem = square baled alfalfa hay fed directly from small square bales.

Table 2 Average intake and digestibility measures of long stem and chopped alfalfa hay treatments fed to crossbred lambs.

	Treatment			*P*-value
Item	Chopped hay[1]	Long stem hay[2]	SE	
DMI, kg•lamb^{-1}•d^{-1}	1.97	1.85	0.104	0.43
OM intake, kg•lamb^{-1}•d^{-1}	1.80	1.64	0.093	0.26
DM digestibility, %	47.23	46.56	0.011	0.68
NDF, digestibility,%	39.94	36.70	0.017	0.20
OM digestibility,%	49.39	47.99	0.011	0.37

[1] [1]Chopped hay = square baled alfalfa hay ground to pass a 2.54 cm screen (Art's Way 5105).
[2] Long stem = square baled alfalfa hay fed directly from small square bales.

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EFFECTS OF POST-AI NUTRITION IN FERTILITY OF YEARLING BEEF HEIFERS

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ABSTRACT: The objective of these experiments was to determine the effects of nutrient restriction for 21 d following AI on fertility of yearling beef heifers. Experiment 1 was conducted at the University of Wyoming using 14 mo old Angus-cross heifers (n = 58; initial BW = 385 ± 31.7 kg; initial BCS = 5.31 ± 0.31). Prior to trial initiation, heifers were developed in a dry-lot and fed a common diet to gain approximately 65% of their mature BW. Heifers were enrolled in the 7-d CO-Synch + CIDR protocol and fixed time-AI (TAI). Immediately following TAI, heifers were blocked by BW and randomly assigned to one of two dietary treatments for a 21 d experimental period: 1) formulated to meet NRC (2000) requirements for heifers to continue gaining at a rate equal to that pre-breeding (GAIN), and 2) formulated to meet NRC nutrient requirements for maintenance only (MAINTAIN). Estrous detection was conducted following TAI and a second service was performed on heifers observed in estrus. Blood samples were collected at d 6, 10 and 14 after TAI, and analyzed for serum concentrations of IGF-1 and plasma concentrations of progesterone. Pregnancy rates were determined via ultrasonography on d 35 and d 65 after TAI. Experiment 2 was a large field trial conducted on 728 commercial Angus heifers developed to approximately 65% of their mature BW in a dry-lot, from which 261 heifers stayed in the dry-lot for a 30 d period and the remaining 467 were transported to a pasture immediately following AI. Data were analyzed using the MIXED and GLIMMIX procedures of SAS. As expected, ADG and change in BW were greater ($P < 0.01$), and final BW tended to be greater ($P = 0.08$) for heifers fed the GAIN diet compared to MAINTAIN. The GAIN (72.4%) treatment approached a tendency ($P = 0.18$) to have greater timed-AI pregnancy rates than the MAINTAIN (55.2%) treatment. Consistent with the treatments, average concentrations of serum IGF-1 tended to be greater ($P = 0.10$) for heifers on the GAIN diet compared to the MAINTAIN. Plasma progesterone concentrations were also greater ($P < 0.01$) for heifers on the GAIN diet on d 10. In Experiment 2, heifers that remained in the dry-lot for a 30 d period following AI had greater AI (67.4 vs. 59.5%; $P = 0.03$) and overall (88.5 vs. 77.5%; $P < 0.01$) pregnancy rates when compared to those moved to pasture. These results suggest that failure to maintain the pre-breeding nutritional plane during the 21 d immediately following AI can marginally reduce conception rates in yearling beef heifers.

Keywords: nutrient restriction, post insemination

Introduction

Replacement heifers represent a significant cost to the beef cow/calf enterprise. In order to maximize productivity, replacement heifers need to be sexually mature at 12 mo of age so they can calve by 24 mo of age (Patterson et al., 1992). Moreover, nutritional requirements from weaning to breeding that ensure pubertal attainment have been extensively investigated. Little information is available, however, on how nutrition immediately following breeding may impact reproductive success. Furthermore, there is evidence that nutritional stress of yearling heifers post-AI may affect reproductive competence as much as pre-breeding nutritional stress (Ashworth et al., 2009; Endecott et al., 2013). This becomes particularly important for operations where heifers are developed in a dry-lot type environment and moved to spring pastures after AI, or at the beginning of the breeding season (Perry et al., 2009; Funston et al., 2012). Such change in nutrition may negatively impact metabolism, body weight gains, and ultimately reproductive efficiency in these beef heifers. Many endocrine and physiological functions, important for conceptus development and survival, may be affected by plane of nutrition during early gestation (Bridges, 2012). To that extent, we have previously demonstrated (Arias et al., 2012) that heifers failing to maintain body weight gains following insemination had reduced AI pregnancy rates. Therefore, the hypothesis of the current study was that decreasing the plane of nutrition immediately following AI would result in decreased pregnancy rates. Our specific objectives were to evaluate how a change in nutritional intake following AI would affect pregnancy success and evaluate hormonal mediators, IGF-1 and progesterone that are known to impact fertility in cattle.

Materials and Methods

All procedures were approved by the University of Wyoming Animal Care and Use Committee.

Experiment 1 (Exp. 1): Animals and experimental design. Angus-cross heifers (n = 58; initial BCS = 5.31 ± 0.31) at the University of Wyoming were developed in a dry-lot to approximately 65% of their mature BW. The project was conducted using a randomized complete block design. Heifers were sorted by initial BW (385 ± 31.7 kg) into BW blocks (blocks 1 to 3). One of 2 dietary treatments was then randomly assigned to 1 of 3 pens (9 or 10 heifers/pen) within each block immediately following routine estrous synchronization and AI.

Dietary treatments and estrous synchronization. Estrous synchronization was accomplished using the 7-day CO-Synch + CIDR protocol and all heifers were timed-AI (TAI) at 54 hours of CIDR removal, considered to be experimental d 0. Immediately following AI, heifers received one of two dietary treatments (Table 1). Half of the heifers were fed a diet formulated to allow gains at a rate identical (0.68 kg/day) to that prior to initiation of trial (**GAIN**). The remaining heifers were offered a diet formulated to meet nutrient requirements (NRC, 2000) for maintenance (**MAINTAIN**). Dietary treatments were maintained for 21 d following AI. Following the initial TAI, estrous detection was conducted and heifers observed in estrus were inseminated. Following the 21 d treatment period, all heifers were commingled and placed on pasture for the remainder of the grazing season. Pregnancy success to TAI was determined 35 d after timed-AI via ultrasonography. Overall breeding season pregnancy rates were determined 65 d after the first AI.

Sampling and laboratory analyses. Blood samples (6 mL) were collected via coccygeal venipuncture on days 6, 10 and 14. Samples from d 6 and 10 were analyzed for progesterone to evaluate progesterone concentrations on maintenance of pregnancy with changes in nutrition; Samples from d 6 and 14 where analyzed for IFG-1 to assess nutritional status after AI. After collection, samples were allowed to clot on ice and were centrifuged within 3 h of collection at 1,500 × *g* at 4°C for 20 min. Serum and plasma were harvested and stored at −20°C until determination of IGF-1 and progesterone respectively. Plasma samples were assayed for progesterone concentration using a commercially available RIA kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA). Concentrations of IGF-1 in serum were determined in duplicate using RIA after an acid–ethanol extraction procedure (Funston et al., 1995).

Experiment 2 (Exp.2): *Animals, experimental design, and treatments.* This was a large field trial conducted on 728 commercial Angus heifers developed to approximately 65% of their mature body weight in a dry-lot in Wheatland, WY. Estrous was synchronized using the MGA-PG protocol. Estrous expression was assessed for 72 h following $PGF_{2\alpha}$ administration. Heifers observed in estrus were inseminated based on the AM/PM rule. Heifers not observed in estrus were TAI at 72 h following $PGF_{2\alpha}$ administration, concurrent with GnRH administration. Following estrous synchronization, 261 heifers stayed in the feedlot for a 30 d period while the remaining 467 were transported to a ranch in Evanston, WY (365 mi) 3 days after the conclusion of AI and placed on spring pastures with an additional 45 d of bull exposure. Artificial insemination and overall pregnancy rates of heifers were determined 35 and 75 d after timed-AI respectively via ultrasonography.

Statistical analysis. Continuous dependent variables including final BW, change in BW, final BCS, change in BCS, ADG, blood serum IGF-1 concentrations and blood plasma progesterone concentrations were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Categorical variables including AI pregnancy, 2nd service conception, and overall breeding season pregnancy rates were analyzed using the GLIMMIX procedure of SAS for binary variables. The models were tested for the fixed main effects of treatment and block and their interaction, neither block nor block × treatment were significant ($P > 0.10$) and were removed from the model. Differences were considered to be significant when $P \leq 0.05$ and a tendency when $0.05 < P \leq 0.10$.

Results and Discussion

As expected, ADG (Table 2) was greater ($P < 0.01$) for heifers fed the GAIN diet (0.47 kg/d) compared to the MAINTAIN diet (-0.28 kg/d), where heifers lost weight. Similarly, change in BW was greater ($P < 0.01$), and final BW tended ($P = 0.08$) to be greater for heifers on the GAIN diet. No differences were detected for BCS and change in BCS ($P > 0.10$) between the two treatments. These results are similar to those obtained on our previous study, except in Arias et al. (2012) the GAIN treatment also resulted in an increase BCS change ($P < 0.01$).

There was a 17.2 percentage point numerical difference (Table 3) in TAI pregnancy rates between the GAIN (77.4%) and MAINTAIN (55.2%) treatments that only approached tendency ($P = 0.18$), likely due to the low number of experimental units (n = 28/treatment). In a similar fashion, there was an 8.7 percentage point difference ($P = 0.70$) in second service pregnancy rates, and a 10.3 percentage point difference ($P = 0.29$) in overall pregnancy rates. Nonetheless, these results are consistent with previous findings from studies conducted at the University of Wyoming and Purdue University where heifers that gained BW following AI had greater first service ($P = 0.02$), second service ($P = 0.06$), and overall ($P = 0.01$) pregnancy rates when compared to heifers that either maintained or lost BW during the 21 d post-AI period (Arias et al., 2012). Similarly, Perry et al. (2009) evaluated pregnancy success rates on heifers moved from a dry-lot to spring grazing after AI compared to those that remained in the dry-lot 42 d after breeding. When heifers were developed in dry-lot, pregnancy success tended to increase with an increase in weight gain after moving heifers to grass. Also in accordance with these results, heifers from Exp. 2 that remained in the dry-lot for a 30 d period following AI had greater AI (67.4 vs. 59.5%; $P = 0.03$) and overall (88.5 vs. 77.5%; $P < 0.01$) pregnancy rates when compared to those moved to pasture immediately following AI. According to Perry et al. (2010), shipping stress can have a significant impact on embryo survivability during

the most critical points of development (blastocyst formation, hatching, maternal recognition of pregnancy and attachment to the uterus). However, all these events take place from d 5 and 42 of gestation, before d 5 the embryo is in the oviduct and is not subject to changes in the uterine environment (Geary, 2006). In Exp. 2, heifers were transported prior to day 5 of gestation; therefore, nutritional status is believed to have influenced the difference in pregnancy rates rather than shipping stress. A recent study by Kruse et al. (2013) examined the effects of post-AI nutrition on day 6 embryo quality. The design of their study was similar to the current study, with heifers fed either to gain or to lose weight following AI. The authors reported that embryos collected from heifers that were fed to lose weight after AI had a reduction in embryo stage and quality ($P < 0.05$), total blastomeres ($P = 0.03$), and percentage of live cells ($P = 0.01$) compared to embryos collected from heifers fed to gain weight following AI.

Concentrations of IGF-1 on day 6 after AI and overall serum IGF-1 concentrations of heifers in Exp. 1 tended to be greater ($P \leq 0.10$) for heifers on the GAIN compared to those in the MAINTAIN treatment. Since plasma concentrations of IGF-1 are positively correlated with body condition and nutrient intake (Houseknecht et al., 1988; Elsasser et al., 1989; White et al., 2001), lower IGF-1 levels were expected for heifers in the MAINTAIN treatment, reflecting their decreased plane of nutrition. It has been demonstrated that reduced plasma IGF-1 concentrations in cattle have a negative effect on fertility (Leroy et al., 2008; Webb et al., 2004). IGF-1 induces mitosis and prevents apoptosis in bovine embryos (Velazquez et al., 2011); therefore a reduction in serum IGF-1 post AI may be one reason for reduced fertility observed in this and similar studies.

In addition, progesterone concentrations were decreased in the MAINTAIN compared to the GAIN treatment on d 10 after AI. Reduced progesterone concentrations during early gestation have been associated with a reduced conceptus growth, lesser production of interferon tau, and greater incidence of pregnancy failure (Mann and Lamming, 2001).

Implications

Results from this study suggest that circulating concentrations of progesterone and IGF-1 are influenced by changes in the nutritional plane of heifers during the 21 d following AI. Both of these hormones can affect the establishment and maintenance of pregnancy in cattle. In addition to progesterone and IGF-1, more research is necessary to determine if synthesis and secretion of other metabolic hormones such as insulin, IGF binding proteins, growth hormone, and leptin are affected by nutritional changes during this critical period of pregnancy and what effect they have on early embryo development. To maximize reproductive success, beef heifers should be managed to allow for a positive nutritional plane that results in weight gain during at least the first 21 d after insemination.

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Table 1. Formulated ingredient and chemical composition of diets fed to yearling beef heifers (Exp. 1).

	Treatments[1]	
Item	GAIN	MAINTAIN
DMI, kg	8.5	7.0
Ingredient, %		
Grass hay	94.2	99.6
Distillers grain	5.4	-
Mineral supplement	0.4	0.4
Chemical composition		
DM, %	78.9	89.9
NEm Mcal/kg	1.29	1.23
NEg Mcal/kg	0.72	0.67
CP, %	9.4	8.3
TDN, %	59.5	57.4

[1]GAIN: diet formulated to meet NRC (2000) requirements for heifers to gain weight, and MAINTAIN: maintenance diet formulated to meet NRC nutrient requirements for maintenance.

Table 2. Effect of post-AI nutrition on growth changes in yearling beef heifers (Exp. 1).

	Treatment[1]			
Item	GAIN	MAINTAIN	SEM[2]	*P* - value
Initial BW	385.4	384.6	6.18	0.93
Final BW, kg	394.2	378.6	6.54	0.08
Change in BW, kg	9.93	-6.00	1.64	<0.001
ADG, kg	0.47	-0.28	0.08	<0.001
Initial BCS	5.27	5.34	0.06	0.45
Final BCS	5.68	5.68	0.06	0.95
Change in BCS	0.41	0.34	0.06	0.45

[1]GAIN: diet formulated to meet NRC (2000) requirements for heifers to gain weight, and MAINTAIN: maintenance diet formulated to meet NRC, 2000 nutrient requirements for maintenance.
[2]The greatest SEM is presented (n = 29/treatment).

Table 3. Effect of Post-AI nutrition on reproductive performance and circulating serum IGF-1 and plasma progesterone in yearling beef heifers (Exp. 1).

	Treatment[1]			
Item	GAIN	MAINTAIN	SEM[2]	*P* - value
Pregnancy success rate, %				
Timed-AI	72.4 (21/29)	55.2 (16/29)	n/a	0.18
Second service	62.5 (5/8)	53.8 (7/13)	n/a	0.70
Overall	89.6 (26/29)	79.3 (23/29)	n/a	0.29
Hormonal mediators, ng/mL				
IGF-1 at day 6	161.2	144.7	6.68	0.07
IGF-1 at day 14	138.9	135.0	4.79	0.52
IGF-1 average	150.0	139.2	4.85	0.10
Progesterone at day 6	2.32	2.46	0.35	0.74
Progesterone at day 10	6.92	4.21	0.60	<0.001

[1]GAIN: diet formulated to meet NRC (2000) requirements for heifers to gain weight, and MAINTAIN: maintenance diet formulated to meet NRC, 2000 nutrient requirements for maintenance.
[2]The greatest SEM is presented (n = 29/treatment).

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OPTIMUM PERFORMANCE TESTING PERIODS FOR DETERMINING FEED EFFICIENCY AND ITS PARAMETERS IN BEEF CATTLE

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ABSTRACT: The Beef Improvement Federation (BIF) recommends that residual feed intake (RFI) be calculated after animals have been on a feed intake test for 70 d with a 21 d pre-test adjustment period. Individual feed intake and gain measurements necessary to calculate RFI are expensive, time consuming, and labor intensive; and therefore limit collection of intake data. If a shortened test length results in similar animal rankings, there would be opportunity to cheapen costs of data collection on a per animal basis. The experimental design was a longitudinal study to determine 1) if average daily intake (ADI) predicted prior to 70 is strongly correlated with and predictive of 70 d measures and 2) if RFI estimates from a shortened test predict and correlate well with results from a 70 d test. We hypothesized that RFI and ADI estimates calculated on shorter performance tests will be strongly correlated to those from a BIF recommended 70 d test period. Using feed intake and weight measures from post-weaning feedlot *Bos taurus* bulls, steers and heifers (n = 615) at Colorado State University's Feed Intake Unit (FIU) from four 70 d performance tests, RFI values for each animal were calculated using the regression of average daily intake (ADI) on metabolic mid-weight (MMWT) and ADG. Performance data and ADI was computed at test days 14, 28, 42, 56, and 70. These measures were used to calculate MMWT and ADG for each two-week period within test. Using the GLM procedure in SAS, values for d 70 RFI and ADI were regressed on d 14, 28, 42, and 56, respectively. The effects of test and sex were also included in each model. Estimated coefficients obtained from the regression of d 70 RFI on d 14, 28, 42 and 56 RFI values were 0.51, 0.77, 0.96, and 1.00, respectively. In addition, Pearson correlation coefficients (r) were 0.55, 0.67, 0.80 and 0.87, respectively. Likewise, estimates for regression coefficients of d 70 ADI on d 14, 28, 42 and 56 ADI values were 0.70, 0.91, 1.01, and 1.03, with Pearson correlations of 0.88, 0.94, 0.97 and 0.99, respectively. Although more research is needed, RFI values from a 56 d test ($P < 0.0001$) and ADI values from a 42 d test ($P < 0.0001$) were shown to predict RFI and ADI at test d 70 in a linear fashion. These results suggest that shorter testing periods to determine RFI and ADI could ultimately reduce testing costs, result in collection of data on larger number of animals per year and allow producers to make selection decisions sooner.

Key words: residual feed intake, average daily intake, beef cattle

Introduction

Rising feed costs have caused producers to become increasingly interested in improving feed efficiency in beef cattle. Feed costs represent approximately one-half the total cost of production for producers (Kennedy et al., 1993). As a result, residual feed intake (RFI) has become a trait of interest for measuring feed efficiency (Kennedy et al., 1993). Residual feed intake is the difference of predicted average daily intake adjusted for performance and the actual average daily intake for an animal. A 21-d adaptation period is recommended to allow animals to adjust to facilities and test ration, followed by a 70-day testing period to determine the rate of gain and daily intake (BIF 2010). Individual animal feed intakes are necessary to calculate RFI estimates but are expensive, labor intensive and time consuming to collect (Wang et al., 2006). Shorter performance tests could result in up to 20% reduction in cost based on historical Colorado State University charges and would to allow facilities to test more individuals in a year. The objectives of this study were to determine 1) if average daily intake (ADI) from a shorter test periods was predictive of standard 70 d values and 2) the impact of these shorter test periods on the resulting RFI calculations. We hypothesized that the RFI and average daily intake

(ADI) estimates calculated from shorter performance tests would be strongly correlated to those from a 70-d testing period.

Materials and Methods

Animal procedures. All animal procedures were approved by the Institutional Animal Care and Use Committee at Colorado State University. Data was compiled from four performance tests (contemporary groups 1-4) at Colorado State University's Feed Intake Unit (**FIU**) located at the Agriculture Research, Development and Education Center (ARDEC) in Wellington, CO. The four tests were conducted over two years: May to July 2011, December 2011 to February 2012, May to July 2012 and September to November 2012. The average daily gain (ADG), weight, and average daily dry matter intake for d 0, 14, 28, 42, 56 and 70 was measured on 615 post weaning bulls, steers and heifers (n = 472, 120 and 23, respectively). Breeds for each contemporary group are detailed in Table 1. The FIU is equipped with the GrowSafe Feed Intake monitoring system (GrowSafe Systems, Ltd., Airdire, Alberta, Canada). The FIU is comprised of 6 pens housing up to 35 cattle per pen and is equipped with 4 GrowSafe nodes per pen. For each performance test, animals were tagged with an electronic ear tag (EID; Allflex USA Inc., Dallas TX) and weighed before entry into the FIU for a 21 d adaptation period followed by the 70 d performance test. Cattle were weighed (d 0) after adaptation and individual feed intakes were measured for 70 d. Cattle were fed *ad libitum.* Rations (Table 2) were modified between each test based on the commodities available with NEg ranging from 47.5 to 52 Mcal/cwt and CP ranging from 13.37 to 14.5%. During the 70 d test, weights were collected every 14 d .

Statistical analyses: For each weigh day (d 14, 28, 42, 56 and 70) average daily dry matter intake (ADI), metabolic mid-weight (MMWT) and average daily gain (ADG) were calculated. Average daily gain was calculated using a linear regression of weight against day of test. Metabolic mid-weight was determined by the mid-test body weight raised to the power of 0.75. Using the REG procedure in SAS (SAS Institute Inc., Cary, NC), ADI was regressed on MMWT and ADG for d 14, 28, 42, 56 and 70 and individual RFI was determined as the difference between individual actual feed intake and expected feed intake. The longitudinal study was conducted by using the GLM procedure in SAS to regress ADI and the resulting RFI values for d 70 on the ADI and RFI for d 14, 28, 42, and 56 with the effect of sex and contemporary group included in the model. The CORR procedure in SAS was used to determine Pearson correlations for d 70 ADI and RFI values to d 14, 28, 42, and 56 values. Spearman rank correlations were also calculated to investigate potential changes for d 70 ADI and RFI compared to d 14, 28, 42, and 56 values.

Results and Discussion

Summary statistics are presented in Table 3. Results from the regression of d 70 ADI on d 14, 28, 42 and 56 ADI are shown in Table 4. As expected, when the shortened test periods approach the benchmark 70 day test the regression coefficients were near one. When d-70 was regressed on d-42 ADI the resulting regression coefficient was 1.01 ($P < 0.0001$) with an R^2 of 0.96 and a Pearson correlation of 0.97. The Spearman rank correlation at d 42 was 0.97 which demonstrates little change in ranking of cattle for ADI. These results would indicate that d-42 intakes are essentially equivalent predictors of ADI from a 70-d and a 42 d test would be sufficient for accurate measurement of ADI.

The benefit of shorter testing periods for ADI has been reported in the literature by Archer et al. (1997) and Wang et al. (2006). Both studies recommended shortening tests to 35 days for the collection of ADI data. These tests both analyzed the variance of ADI every 7 days during at 91 d and 119 d performance test. Wang et al. (2006) analyzed ADI using a repeated measures model which allowed for heterogeneous variance and correlations among different time intervals on test. In both studies they found that the variance for ADI stabilized on d 35. Archer and Bergh (2000) examined feed intake for *Bos taurus* and *Bos indicus* cattle and found, using variance components, that they could shorten feed intake tests to 56 d and argued that the test could be shortened to 42 d without significant loss of accuracy which support our findings.

Results from the regression of d-70 RFI on d 14, 28, 42 and 56 RFI are shown in Table 4. As with ADI, the regression coefficients for RFI values increased as the shortened test period neared the benchmark 70 d test. The regression coefficient of d 70 regressed on d 56 resulted in a regression coefficient of 1.00 ($P<0.0001$) with an R^2 value of 0.76 and a Pearson correlation of 0.87. The Spearman rank correlation for d 56 was 0.86 which indicated some change in rank of animals based on their RFI values. These results suggest that on d-56 of a performance test we could predict the RFI values that would result from a 70 d test.

Most studies on test duration required for RFI computation show results for tests of 70 d or longer (Archer et al., 1997, Archer and Bergh 2000, Wang et al., 2006). All these tests further show that the variance for RFI stabilizes at about 70 d on test but Archer and Bergh (2000) argued that shortening the test to 49 days would result in a minor loss of accuracy.

Our results indicate that shortening the test to d 56 would result in a reliable phenotypic RFI value.

Although RFI is considered a trait of interest for feed efficiency, it is criticized by producers since slow-growing animals eating relatively small amounts of feed may actually have a desirable or lower RFI value (Berry and Crowley, 2012). Studies such as Archer et al. (1997), Archer and Bergh (2000) and Wang et al. (2006) indicate that feed intake can be accurately measured on shorter tests but longer tests are required for calculating ADG and therefore RFI. Therefore, more research on growth rates and their effects on RFI should be considered.

Implications

Because daily feed intake is an economically relevant trait but expensive to measure, alternative approaches to collecting individual performance is needed.

The results from this study suggest that performance tests shorter than 70 days could determine reliable RFI and ADI values and thereby reduce costs and increase number of animals evaluated.

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Table 1. Contemporary group age, sex and breed

CG[1]	Age (d)[2]	Sex	Breed	n
1	414	Bull	Red Angus	33
			Red Angus cross[3]	17
			Angus	4
			Angus cross[4]	3
			Simmental cross	1
			Stabilizer[5]	128
2	278	Heifer	Angus	15
			Hereford	8
		Bull	Angus	81
			Red Angus	20
			Hereford	13
			Angus X Hereford	1
			South Devon X Simmental	43
3	413	Steer	Red Angus	50
		Bull	Red Angus	12
			Red Angus cross	1
			Angus	17
			Angus cross	17
			Simmental cross	4
			Gelbvieh cross	5
			Stabilizer	72
4	539	Steer	Red Angus	70
Total				615

[1]Contemporary group (CG) = performance test

[2]Age = mean age of animals at the start of the test in days

[3]50% to 87% Red Angus

[4] 5/8 or > Angus

[5]Compostie of ≥ 3 or more breeds

Table 2. Composition of rations fed to animals of each contemporary group in the Feed Intake Unit

CG[1]	Sex	Corn Silage	Alfalfa Hay	Grass Hay	Wheat Straw	Whole Corn	Cracked Corn	DDG[2]	Calcium Carbonate	Supplement[3]
1	Bulls	52.44%	23.62%	5.05%	-	17.89%	-	-	-	1.00%
2	Bulls, Heifers	52.07%	17.68%	4.25%	-	25.00%	-	-	-	1.00%
3	Bulls	44.58%	22.42%	-	2.00%	30.00%	-	-	-	1.00%
3	Steers	10.00%	6.90%	-	-	-	74.46%	3.79%	0.75%	4.10%
4	Steers	10.00%	6.90%	-	-	-	74.46%	3.79%	0.75%	4.10%

[1]Contemporary Group (CG) = performance test

[2]DDG = Dry Distillers Grain

[3]Supplement = CSU developer supplement

Table 3. Summary statistics for average daily intake (ADI), metabolic mid-weight (MMWT) and average daily gain (ADG) for all contemporary groups combined (n=615)

	ADI		MMWT		ADG	
Days on Test	Mean	SD[1]	Mean	SD	Mean	SD
d 14	11.17	2.70	75.10	9.54	1.80	1.11
d 28	11.10	2.44	76.73	9.73	1.74	0.73
d 42	11.22	2.44	78.64	10.11	2.08	0.77
d 56	11.23	2.32	79.47	9.71	1.48	0.50
d 70	11.36	2.27	80.75	9.62	1.43	0.46

[1]Summary statistics for all tests combined

[2]SD = Standard deviation

Units are in kilograms

Table 4. Regression Coefficients, R^2, and correlations for average daily intake (ADI) and residual feed intake (RFI)

	ADI[1]				RFI[2]			
d 70 regressed on:	Estimates[3]	R^2	Pearson[4]	Spearman[5]	Estimates	R^2	Pearson	Spearman
d 14	0.7	0.84	0.88	0.91	0.51	0.31	0.55	0.62
d 28	0.91	0.91	0.94	0.95	0.77	0.46	0.67	0.72
d 42	1.01	0.96	0.97	0.97	0.96	0.64	0.8	0.83
d 56	1.03	0.98	0.99	0.99	1.00	0.76	0.87	0.86

[1]ADI = Average daily intake

[2]RFI = Residual feed intake

[3]Estimates = the regression coefficient for d 70 regressed on test day.

[4]Pearson = Pearson's correlation

[5]Spearman = Spearman's rank correlation

All p-values were significant ($p<0.0001$)

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USE OF A PORTABLE METABOLIC CHAMBER FOR MEASURING RESTING METABOLIC RATES IN CATTLE OF VARYING BODY WEIGHTS.

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ABSTRACT: The purpose of this pilot study was to develop a repeatable indirect calorimetry procedure for measuring resting metabolic rate (MR) of cattle of varying BW. We hypothesized BW would be linearly associated with MR. Animals used in this study were two sets of Holstein bull calves (n = 6) and Angus × Hereford heifers (n = 3). Cattle were categorized into three weight groups: heavy, middle, and light (average BW = 322 ± 31; 167 ± 9; and 72 ± 4 kg, respectively). Heavy cattle received a forage-based diet, middle cattle a concentrate-based diet, and light cattle, milk replacer plus concentrate. Resting MR was measured in a portable chamber using the Field Metabolic System (Sable Systems International, Las Vegas, NV). The metabolic chamber was a horse trailer (8.3 m^3) connected to a computerized (Expedata) pull mode respirometer capable of measuring O_2 consumption (V_{O2} - L/min), and CO_2 production (V_{CO2} - L/min). Flow rate was set at 1,000 L/min. Individual animals were confined to the trailer for a 30 minute period or longer, until the animal calmed down. Animals were measured twice for V_{O2}, V_{CO2}, and BW, on 28-Feb-2013 (Run 1) and 7-Mar-2013 (Run 2). Respiratory quotients (RQ = V_{CO2}/V_{O2}) were calculated for each animal per run. The correlation between repeated V_{O2}, V_{CO2}, and BW measurements were 0.89, 0.91, and 0.999. Average RQ for Runs 1and 2 were 0.75 L/min and 0.74 L/min, respectively. The respiratory quotient reflects the animal's diet at a resting metabolic rate and is expected to range from 0.70-1.00 L/min. A value near 0.70 L/min represents lipid catabolism and a value near 1.00 L/min represents carbohydrate catabolism. Using a paired t-test, RQ did not differ between Runs 1 and 2 (P = 0.89). The relationship between an animal's BW and MR was explained by the equation: $V_{O2} = 0.0173 \times BW^{0.8037}$ ($P <$ 0.01). This portable metabolic chamber consistently measured resting metabolic rate of these cattle.

Key words: resting metabolic rate, cattle, portable metabolic chamber

Introduction

Metabolic rate (MR) can be measured using indirect calorimetry, a procedure that measures rate of inhaled O_2 and respired CO_2. Determining metabolic rate may be used to quantify energy expenditure. In beef production, one of the earliest uses for calorimetric procedures was to determine maintenance energy requirements for growing and finishing cattle (ARC, 1965; NRC, 2000). Indirect calorimetry historically required confinement chambers that were immobile and laboratory-controlled (Kelly et al., 1993). This limited the number of cattle that could be measured feasibly and restricted the number of extrinsic factors that could be assessed (Kelly et al., 1993; NRC, 2000). Today, a number of companies provide calorimetric equipment that can be used with different confinement chambers, making it possible for researchers to take their research to the field. Measuring resting metabolic rate in a field setting accounts for an animal's response to the local environment, current diet, and season.

Using a commercially available, open-flow respirometer, we modified a horse trailer into a chamber capable of determining rate of O_2 consumed and CO_2 produced by individual animals. We were particularly interested in developing a method that was repeatable and applicable in a field setting. Therefore, the primary objectives of this study were to: 1) measure the correlation between repeated metabolic measurements on individual animals and 2) to assess the scaling of metabolic rate for cattle of varying body weights. Our null hypotheses were that repeated metabolic measurements were not correlated and that metabolic rate of cattle did not vary by individual BW.

Materials and Methods

Animals

In the late winter of 2013, two sets of Holstein bull calves (n = 6) and Angus × Hereford heifers (n = 3) were used to determine resting metabolic rate. Cattle were randomly selected from three groups of research animals at the Bozeman Agricultural Research and Teaching Farm in Bozeman, MT. Body weights of these animals represented three categories: heavy (average BW = 322 ± 31 kg), middle (average BW = 167 ± 9 kg) and light (average BW = 72 ± 4 kg). Cattle in all three weight categories were receiving different diets: heavy cattle, a forage-based diet (alfalfa hay), middle cattle, a concentrate-based diet (88% barley, 10% alfalfa-hay, and 2% supplement), and light cattle, milk replacer (Nurture Pinnacle, Provimi North America Inc., Brookville, OH) plus ad libitum concentrate diet (Payback Dairy Calf Starter, CHS Nutrition, Sioux Falls, SD).

Indirect Calorimetry Procedure

Metabolic rate of individual animals was measured on 28-Feb-2013 (Run 1) and 7-Mar-2013 (Run 2) with a portable metabolic chamber connected to a Field Metabolic System

(Sable Systems International, Las Vegas, NV). The metabolic chamber was a horse trailer (8.3 m^3) adapted with a computerized (Expedata) open-flow respirometer capable of measuring O_2 consumed (V_{O2} in units of L/min), and CO_2 produced (V_{CO2} in units of L/min; Figure 1 and 2). Cattle were confined to the trailer individually. Metabolic rate was measured over a 30 minute period or until the animal had settled down, which sometimes took up to 45 min. For Run 1, the trailer was pulled within a heated shop (12°C ± 3). The main door was left partially open for the exchange of air. For Run 2, the trailer was parked outside (1°C ± 2). Metabolic rates were sampled between 900 h and 1800 h. Animals were weighed without fasting before they were loaded into the trailer.

Figure 1: Horse trailer adapted as an indirect calorimetry system for measuring rate of oxygen consumed and carbon dioxide produced by cattle.

Figure 2: Field metabolic system (Sable Systems, Las Vegas, NV, USA) hooked up to a horse trailer and used for measuring rate of oxygen consumed and carbon dioxide produced for cattle confined to a horse trailer.

The Field Metabolic System sampled air continuously, alternating between ambient air (outside the chamber), serving as a baseline, and air pulled from the chamber. Flow rate was set at 1000 L/min (Sable Systems International, 2010). A subsample of respired air (1 mL) was passed through the column where the flow rate, water vapor, CO_2, and O_2 were measured. Readings were monitored every 1s by the gas-analyzer and recorded in the Expedata program. The data program outputted rates of CO_2, and O_2 in L/min, which were used to calculate the respiratory quotient (RQ = V_{CO2}/V_{O2}). Respiratory quotients were calculated for each animal per run. Metabolic rate in L/min was represented by the rate of O_2 consumed (Lighton, 2008).

Statistical Analysis

Pearson's correlation coefficient was used to determine if BW, RQ, V_{O2}, and V_{CO2} measurements were correlated between Run 1 and Run 2 (PROC CORR; SAS 9.2, SAS Inst. Inc., Cary, NC). A t-test was used to determine if RQ of individual animals differed between Run 1 and Run 2 (PROC TTEST; SAS 9.2, SAS Inst. Inc., Cary, NC). Linear regression was used to determine if MR was related to BW (PROC REG; SAS 9.2, SAS Inst. Inc., Cary, NC). Differences were considered significant at $P \leq 0.05$.

Results

Correlations of Run 1 and Run 2 for BW, RQ, V_{O2}, and V_{CO2} measurements were 0.999, 0.72, 0.89, and 0.91 (Table 1). Average RQ for Runs 1 and 2 were 0.75 L/min and 0.74 L/min, respectively. Respiratory quotients did not differ between Runs 1 and 2 (P = 0.89). The relationship between an animal's BW and MR was explained by the equation: $V_{O2} = 0.0173 \times BW^{0.8037}$ ($P < 0.01$; Figure 3).

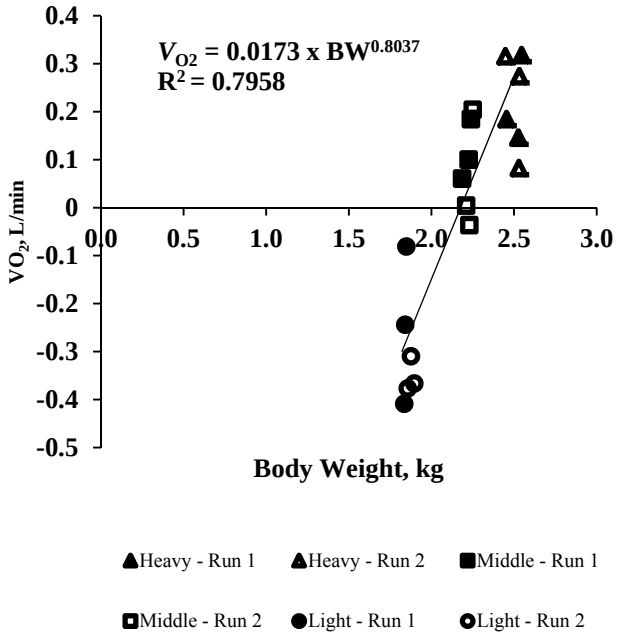

Figure 3: Relationship between body weight and rate of oxygen consumed (VO_2) for individual animals on a logarithmic scale.

Discussion

Our portable metabolic chamber yielded highly correlated metabolic measurements of cattle between Run 1 and Run

2. For instance, RQ measurements for Run 1 and Run 2 did not differ. Respiratory quotients for animals at resting metabolism commonly range from 0.70-1.00 L/min (Lighton, 2008). Respiratory quotients for heavy, middle, and light cattle in this study were within this range: 0.71, 0.78, and 0.75, respectively.

We also assessed the scaling of metabolic rate over cattle of varying body weights. When an animal is at a resting state the energy required to break down body tissues and provide nutrients is known as resting metabolism and is related to the body weight of the animal (Black et al., 1939). Differences in intraspecific metabolic rate can thus be attributed to variation in body mass (Willmer et al., 2000). The relationship between metabolic rate and body mass is known as the Kleiber relationship, and is important for understanding how much of the difference in metabolic rate is attributed to body weight (Kleiber, 1958; Kleiber, 1961). In this study, the mass scaling coefficient was 0.80, so for every 1 kg increase in the animal's BW the rate of oxygen consumption increased 0.80 L/min. This value compares to the standard literature value for metabolic size which is $BW^{0.75}$ (Kleiber, 1961).

Stressed cattle may have higher metabolic rates. Cattle were measured for at least 30 min in the portable chamber. This timeframe was based upon the time it took for the individual animal to settle down. A calm animal was defined as an animal that: 1) laid down, 2) had minimal variability in CO_2 and O_2 trend lines, or 3) demonstrated low O_2 consumption and CO_2 production rates. However given RQ did not differ between sampling dates we concluded 30 min was adequate for this pilot project. To assess the minimum time required to measure metabolic rates we would need to continuously measure CO_2 respired and O_2 consumed over a longer period.

Extreme temperatures affect MR of cattle (Young, 1975; NRC, 2000). Ambient temperatures above the upper critical temperature of cattle increase MR to dissipate heat (NRC, 2000). Ambient temperatures below the lower critical temperature of cattle increase MR to produce heat (NRC, 2000). In both these instances, maintenance requirements of cattle increase in order to maintain a constant body temperature (NRC, 2000). Temperatures differed by 11°C between Run 1 and Run 2. Cattle were acclimated to these temperatures, and the temperatures were likely not above or below their upper or lower critical temperatures. Thus, we do not believe temperature affected MR of these cattle, because the rate of oxygen consumed was highly correlated between Runs 1 and 2.

In this study we were not interested in assessing factors that could affect metabolic rate of cattle. We were interested in determining if our portable metabolic chamber yielded reliable measurements. For this reason, we did not control for the confounding effects of gender, age, and diet. Future studies will need to consider the effect of these factors on resting metabolic rates.

Implications

The mobility of this chamber will make it useful to assess energy metabolism of cattle in a variety of environments.

Acknowledgement

Appreciation is expressed to Provimi North America Inc., Brookville, OH for providing the milk replacer.

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Table 1. Body weight, respiratory quotient, rate of oxygen consumed, and rate of carbon dioxide produced for Holstein bull calves and Angus × Hereford heifers confined and measured in a portable metabolic chamber

Item[1,2,3]	Heavy[4] (n=3)	SEM	Middle[5] (n=3)	SEM	Light[6] (n=3)	SEM	Correlation[7]
BW, kg							
Avg	321.8	12.58	167.4	3.52	72.4	1.62	0.999
Run 1	323.9	20.00	164.8	5.96	69.5	0.59	
Run 2	319.6	19.66	169.9	4.45	75.4	2.03	
RQ							
Avg	0.71	0.03	0.78	0.04	0.75	0.06	0.72
Run 1	0.75	0.01	0.74	0.04	0.76	0.04	
Run 2	0.67	0.04	0.81	0.06	0.75	0.13	
$\dot{V}O_2$, L/min							
Avg	1.70	0.15	1.25	0.11	0.52	0.07	0.89
Run 1	1.67	0.21	1.31	0.11	0.60	0.13	
Run 2	1.72	0.26	1.18	0.21	0.45	0.02	
$\dot{V}CO_2$, L/min							
Avg	1.19	0.10	0.95	0.04	0.39	0.05	0.91
Run 1	1.25	0.17	0.97	0.03	0.45	0.08	
Run 2	1.13	0.12	0.93	0.09	0.33	0.05	

RQ = respiratory quotient
$\dot{V}O_2$ = rate of oxygen consumed (L/min)
$\dot{V}CO_2$ = rate of carbon dioxide produced (L/min)
[1]Avg represented the average metabolic measurements for Run 1 and Run 2.
[2]Run 1 represented metabolic measurements determined after confining cattle individually to the portable metabolic chamber on 28-Feb-2013.
[3]Run 2 represented metabolic measurements determined after confining cattle individually to the portable metabolic chamber on 7-Mar-2013.
[4]Heavy weight cattle (Angus × Hereford heifers) were consuming a forage-based diet consisting of a grass hay mix.
[5]Middle weight cattle (Holstein bull calves) were consuming primarily a concentrate-based diet (88% barley; 10% forage; 2% supplement).
[6]Light weight cattle (Holstein bull calves) were consuming milk replacer, and calf starter.
[7]Pearson's correlation coefficient for Run 1 and Run 2.

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SUPPLEMENTAL LYSINE DOES NOT AFFECT ANIMAL PERFORMANCE, ANTIBODY TITER, OR RECTAL TEMPERATURE IN RESPONSE TO A MODIFIED-LIVE VIRAL RESPIRATORY VACCINE IN NEONATAL HOLSTEIN CALVES[2]

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ABSTRACT: Infectious bovine rhinotracheitis (IBR), caused by bovine herpesvirus-1, is a contributing pathogen to bovine respiratory disease. Lysine has been shown reduce virulence of herpesviruses in felids and humans. Our objective was to evaluate the effects of supplemental lysine on serum IBR antibody titer and rectal temperature in response to a modified-live intranasal (IN) or intramuscular (IM) respiratory-virus vaccination. Sixty-four neonatal Holstein bull calves (7 ± 2 d of age; BW = 37 ± 4.2 kg) were used in a completely randomized design. Calves were fed milk replacer supplemented with either 17 g/d L-lysine monohydrochloride (LYS; 28 calves) or an equivalent amount of casein (CAS; 28 calves) for 42 d. Calves were then vaccinated with either an IN IBR-parainfluenza virus-3 (PI3; Nasalgen, Merck, Summit, NJ) or an IM (IBR-PI3-bovine viral diarrhea type I and II, bovine respiratory syncytial virus; Express 5, AgriLabs, St. Joseph, MO) modified-live vaccine on d 36. A control group (8 calves) received no supplement or vaccination. All calves were housed in individual calf pens (1.2 x 2.1 m). Daily feed intakes were monitored and BW measured weekly. Calves were bled on d 0, 35, 36, 37 and 42. Temperature data loggers were attached to rectal probes and temperatures were recorded every 5 min from d 28 to d 42. No significant differences were determined for average performance, rectal temperature, or IBR antibody titers with either IN or IM vaccinations between LYS and CAS treated calves ($P > 0.10$). However, serum urea nitrogen and the ratio of serum lysine:arginine increased ($P < 0.05$) for LYS compared to CAS calves. These results suggest that supplementing lysine impacts nitrogen metabolism but does not alter the response to IBR vaccination or animal performance in neonatal Holstein calves.

Key Words: Holstein calves, bovine herpesvirus-1, lysine, vaccination

Introduction

The susceptibility of the respiratory tract to bacterial disease following a viral infection is a matter unresolved. Bovine herpesvirus-1 (BHV-1) is one of the foremost infections contributing to bovine respiratory disease (BRD; Nandi et al., 2009). An infection of BHV-1 can lead to infectious bovine rhinotracheitis (IBR), suppressing the immune system and increasing the risk for a secondary bacterial infection (Yates, 1982). Herpes viruses are difficult to eradicate due to the establishment of latency after exposure (Jones et al., 2000; Nandi et al., 2009). Times of stress, such as those experienced during shipping and processing, can stimulate the virus to emerge from latency, inducing disease and further spreading the virus (Pastoret et al., 1982). Bovine herpesvirus-1 is very common; cattle herds of over 400 head may have BHV-1 prevalence of over 85% (Raaperi et al., 2010). Vaccines are readily available for BHV-1 that attempt to provide enough immunity to avoid reactivation of the disease. Although vaccination is common, humoral immunity is not always sufficient and reactivated-induced disease puts cattle at risk for developing BRD (Jones and Chowdhury, 2007).

There is evidence that lysine supplementation decreases the incidence and severity of herpesvirus-associated disease (Griffith et al., 1981; Maggs et al., 2000). *In vitro* studies have shown lysine has a replication-inhibiting capability of the herpes virus (Maggs et al., 2000). Since IBR is the result of a herpes virus infection, there may be an altered response to the common IBR vaccination when lysine is supplemented at concentrations exceeding requirements. The objective of this study was to investigate the effects of a lysine supplementation on a modified-live viral respiratory vaccine.

Materials and Methods

Procedures were reviewed and approved by the Montana State University Agriculture Animal Care and Use Committee (2012-AA01).

Animals, Treatments and Diets

Sixty-four Holstein calves (7 ± 2 d of age; BW = 37 ± 4.2 kg) were used in a completely randomized design. Sixteen calves were purchased from a commercial Montana dairy and were transported (approximately 30 km; 30 min in transit) to the Montana State University facility after

[1]Appreciation is expressed to Dr. Mark Hill with Provimi Nutrition, Minneapolis, MN, for donating the milk replacer for this study.

[2] Mention of a trade name, proprietary product, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. USDA is an equal opportunity provider and employer.

receiving their morning feeding. Forty-eight calves were purchased from a commercial Idaho calf ranch and were transported (approximately 660 km; 6 h in transit) after their morning feeding to the Montana State University facility. Upon arrival, calves were weighed and randomly assigned to treatments including: **LYS**, lysine supplemented, **CAS**, casein supplemented, and **CON,** control (no supplementation). In addition, calves received either an intranasal (**IN**) modified-live viral respiratory vaccination in experiment 1 or intramuscular (**IM**) modified-live viral respiratory vaccination in experiment 2; **CON** calves received no vaccination. Calves were housed in individual pens (1 x 2 m) fitted with feed and water buckets and nose guards to eliminate calf-to-calf contact. Pens were concrete-surfaced covered with rubber mats. Crates were maintained inside at the Bozeman Agriculture and Teaching (BART) Farm with a mean environmental temperature of 15° C.

All calves were fed a commercial milk replacer (Provimi, Nurture Pinnacle, Brookville, OH; DM: 95.7%; CP: 26.6%; crude fat: 18.7%; ash: 6.4%) at 0.45 kg/d twice daily at 0700 and 1900 for 42 d. Milk replacer was mixed at a concentration of 12% powder (as-fed; Milk Master, PolyTank & Polydome, Litchfield, MN). Milk replacer was fed using 2 L nursing bottles (Calf Nurser Bottle, Merrick, Inc., Vadnais Heights, NM) with a rubber teat (Milk Bar Bottle Nipple, Coburn Company, Whitewater, WI) and individual bottle holders in each pen.

Milk replacer was formulated to meet lysine requirements of 17 g/d (Hill et al., 2007). Lysine-supplemented calves (**LYS)** received an additional 17 $g \cdot head^{-1} \cdot d^{-1}$ of L-lysine monohydrochloride (Global Bio-Chem, Hong Kong, China). Casein-supplemented calves (**CAS)** received 17 $g \cdot head^{-1} \cdot d^{-1}$ of casein. Supplements were added directly to individual bottles at a rate of 8.5 g twice daily to total 17 g/d. No lysine or casein supplementation was delivered to control calves (**CON**). After each feeding, bottles and teats were washed with a detergent, bleach, and water mixture.

Calves were offered a commercial starter feed (Dairy Starter Pellet; Payback Feed, Sioux Falls, SD; DM, 97.8%; ADF, 8.9%; NDF, 21.9%; and CP, 19.9%) beginning on d 3 with an initial offering of 0.2 $kg \cdot head^{-1} \cdot d^{-1}$. Amounts were increased to ensure free choice consumption. Daily allotments of grain were fed starting at 0730, daily refusals were weighed prior to feeding. Offered and refused feed was weighed back daily. Each calf was supplied with a water bucket. Water was changed twice daily. Water buckets were washed with a soap, bleach, and water mixture weekly.

Data Collection

Rectal temperatures were constantly recorded by means of an indwelling rectal temperature probe starting on d 28 and ending on d 42. Temperature data loggers were attached to the rectal probes. Temperatures were recorded every 5 min and averaged every h for the duration of the study for statistical analyses.

Calves were vaccinated on d 35 at approximately 1000 via intranasal inoculation in with 2 mL of a modified-live respiratory vaccine containing BHV-1 and PI3 (Nasalgen, Merck Animal Health, Summit, NJ) experiment 1 or intramuscularly with 2 mL of a pentavalent, modified-live respiratory vaccine containing BHV-1, BVDV1a, BVDV2a, PI3 and BRSV (Express 5, Boehringer Ingelheim Vetmedica, Inc., Saint Joseph, MO) in experiment 2. Control calves (CON) received no vaccination in experiments 1 and 2.

Body weights were obtained on d 0, 1, 7, 14, 21, 28, 35, and 42 prior to the 1900 feeding. Grab samples of the grain and milk replacer diet were collected weekly for analysis. Ingredient samples were analyzed for NDF and ADF, CF, fat and CP (Dairy One Laboratory, Ithaca, NY).

Blood samples were collected via jugular venipuncture on d 0, 7, 14, 21, 28, 35, 36, 37, 39 and 42 in vacuum tubes (Kendall Monojet; Covidien, Mansfield, Massachusetts) for all calves; however, only d 0, 28, 35, 36, 37, 39 and 42 were analyzed. Blood collection was conducted prior to the 1900 h feeding. Serum was centrifuged at 2,100 x *g* for 15 min at 20°C and then stored frozen at -20 °C until analyzed. Frozen serum was transported to the Montana State University Veterinary Diagnostic Lab (Bozeman) for determination of serum neutralizing antibodies for BHV-1, PI3, BVDV type I, BVDV type II, and BRSV. Serum samples were analyzed for SUN content by a commercial assay kit (Urea Nitrogen Direct; Cat. # 0580-250; Stanbio Laboratory, Boerne, TX). Additionally, serum samples were analyzed for serum lysine and arginine concentrations by means of mass spectrometry. Electrospray ionization of samples detected amino acid levels through a spectrometer. Concentrations of L-lysine and L-arginine were used to produce a standard curve. Samples were analyzed by calculating area under the curve.

Calculations and Statistical Analysis

All data was analyzed using the Proc Mixed procedures of SAS (SAS Institute Inc., Cary, NC). Each calf was considered the experimental unit. Performance data was analyzed with a model that included treatment, block (experiment) and calf. For temperature, antibody titer data, serum lysine and arginine, and SUN repeated measures data were analyzed with a model that included treatment, day, and treatment x day. Block, treatment, day, and calf were included in the class statement. No treatment x vaccination interaction for antibody titers, SUN, or serum lysine and arginine, therefore, data were averaged across vaccination treatments. Logarithmic transformations were applied to all titer values. Significance was indicated when $P < 0.05$.

Results

No differences ($P > 0.10$) were observed for initial BW, final BW, ADG, DMI or overall G:F across treatments (LYS, CAS, and CON; Table 1). No differences were observed among treatments (LYS, CAS, and CON) in vaccinated calves for IN (Figure 2) or IM (Figure 3), for rectal temperature ($P > 0.10$) or IBR antibody titer levels ($P > 0.10$; Figure 4). No treatment x time interaction was

observed among treatments. Serum urea nitrogen (Figure 5) and lysine:arginine ratio (Figure 6 and 7) was greater for LYS compared to CAS calves.

Discussion

Performance among calves did not differ. These results are similar to Aubry et al. (2001) reporting no difference in daily gain among vaccinated and non-vaccinated Holstein calves. Lack of immune response in the form of a rectal temperature and antibody titer response may have been impacted by maternal antibody presence. Previous research has established maternal antibodies interfere with vaccination response (Brar et al., 1978; Menanteau-Horta et al., 1985; Kimmen et al., 1987). Further, animals that are seropositive as a consequence of maternal antibodies may have an inhibited response to vaccination early in life (Tizard, 2009). This is supported by work from Schipper et al. (1978) reporting one-third of calves failed to develop antibody titers after receiving an initial IBR vaccination. Although vaccinations were administered 4 wk after arrival, maternal antibodies may have reduced the immune response in vaccinated calves (Menanteau-Horta et al., 1985). IBR antibody titers showed no difference among supplemented and control calves, or vaccinated and non-vaccinated calves. This is similar to the findings of Brar et al., (1978) and Schipper et al. (1978). Alternatively, Aubry et al. (2001) reported higher antibody titers in vaccinated calves compared to non-vaccinated calves.

Lysine supplemented calves received double the daily requirements of lysine. This may have resulted in excess circulating lysine, contributing to increased SUN values.

In conclusion, these results agree with previous findings of a lack of response to vaccination in neonatal calves.

Implications

These results suggest that supplemental lysine will not alter febrile response or IBR antibody titer levels after a modified-live respiratory vaccine but will alter SUN and serum lysine:arginine ratios in neonatal calves.

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Table 1. Effect of supplemental lysine on performance of neonatal Holstein calves

Item	Treatment[1] CAS	LYS	CON	SEM[2]
Calves[3]	28	28	8	-
BW, kg				
Initial	36.5	36.7	34.4	1.1
Final	56.9	59.3	52.4	2.6
Performance, d 0 to 42				
ADG, kg	0.45	0.46	0.43	0.07
DMI, kg	0.66	0.70	0.60	0.13
G:F	0.94	0.65	0.69	0.25

[1]Treatments were supplementation: CAS = 17 g/d casein; LYS = 17g/d L-lysine monohydrochloride; CON = no supplementation
[2]Pooled SEM
[3]Death loss of 4 CAS, 8 LYS, and 2 CON calves.

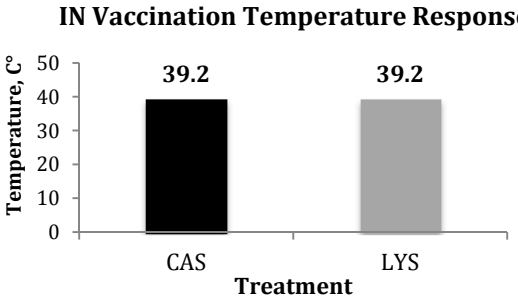

Figure 2. Experiment 1. Effects of IN modified-live IBR-PI3 viral vaccination on average rectal temperature in calves receiving 17g/d of supplemental lysine (LYS) or 17 g/d of supplemental casein (CAS) in neonatal Holstein calves. Pooled SEM (0.08). Averaged rectal temperature was not significant among treatments (P > 0.10).

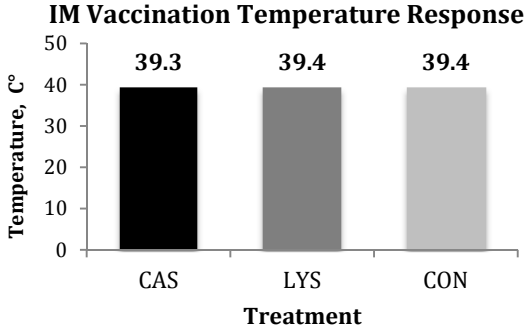

Figure 3. Experiment 2. Effects of IM modified-live IBR-PI3 viral vaccination on average rectal temperature in calves receiving 17 g/d of supplemental lysine (LYS), 17 g/d of supplemental casein (CAS) or no supplementation (CON) in Holstein calves. Pooled SEM (0.08). Averaged rectal temperature did not differ among treatments (P > 0.10).

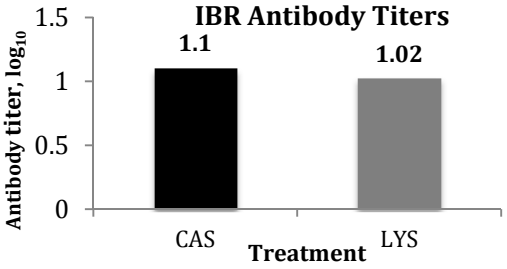

Figure 4. Effects of 17 g/d supplemental lysine (LYS) or 17g/d supplemental casein (CAS) on IBR antibody titers following a modified-live viral respiratory vaccination in neonatal Holstein calves. Antibody titers averaged across experiments. Pooled SEM (0.17). Antibody titers did not differ (P > 0.10) among treatments.

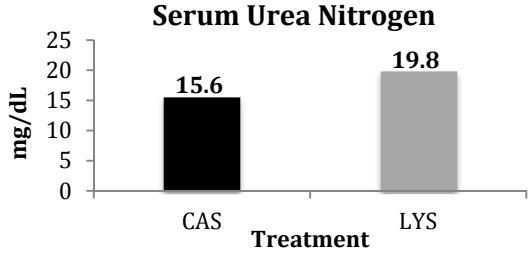

Figure 5. Effects of 17 g/d supplemental lysine (LYS) or 17 g/d supplemental casein (CAS) on serum urea nitrogen in neonatal Holstein calves. SUN averaged across experiments. Pooled SEM (1.47). Overall SUN was greater (P < 0.05) in LYS compared to CAS calves.

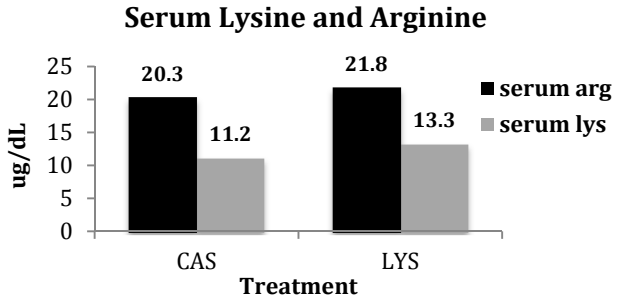

Figure 6. Effects of 17 g/d supplemental lysine (LYS) or 17 g/d supplemental casein (CAS) on serum lysine and arginine in neonatal Holstein calves. Pooled SEM for serum arginine (0.90) and serum lysine (1.18). Serum lysine and serum arginine did not differ (P > 0.10) among treatments.

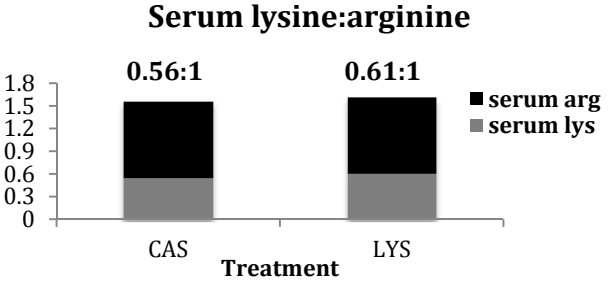

Figure 7. Effects of 17 g/d supplemental lysine (LYS) or 17 g/d supplemental casein (CAS) on serum lysine:arginine ratio in neonatal Holstein calves. Serum lysine:arginine ratio was greater (P < 0.05) in LYS compared to CAS calves.

THE EFFECT OF A 7-D CIDR-BASED PRE-SYNCHRONIZATION PROTOCOL ON RESPONSE TO GNRH AT THE START OF 7-D CO-SYNCH AND PREGNANCY RATE FOLLOWING TIMED AI IN YEARLING BEEF HEIFERS

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ABSTRACT: Our objectives were to evaluate: 1) the effect of using a 7-d controlled internal drug release (**CIDR**) based pre-synchronization protocol on pregnancy rates following fixed-time AI (**TAI**), and 2) ovulation and follicular dynamics after administration of GnRH at the start of CO-Synch + CIDR. Yearling beef heifers (n = 609) at 3 locations were randomly assigned to: 1) 5-d Pre-Synch + 7-d CO-Synch + CIDR, or 2) 7-d CO-Synch + CIDR for a pairwise comparison design. All injections were given intramuscularly at 100 μg of GnRH analogue and 25 mg prostaglandinF2α (**PGF**). On d –14, the 5-d Pre-Synch heifers were administered GnRH and CIDR. The CIDR was removed on d -7 and heifers received an injection of PGF. On d 0, all heifers received a CIDR and GnRH. The CIDR was removed and heifers received PGF on d 7. All heifers received TAI and GnRH 54 ±2 h after PGF. Follicle presence and size were determined in a subset (n = 135) of heifers on d 0 and 3 at 2 locations to determine ovulatory response following GnRH on d 0 via ultrasonography. All heifers were observed for behavioral estrus for 54 hrs following d 7 of synchronization. Pregnancy was determined through ultrasonography 50 ± 5 d after TAI. Body condition scores were collected at all locations (location 1, 5.5 ± 0.70; location 2, 4.8 ± 0.60; location 3, 5.1 ± 0.52) No difference ($P > 0.05$) was found for BCS between locations. No treatment x location interaction ($P = 0.45$) was observed for pregnancy to TAI, and a greater (P = 0.01) proportion of 7-d CO-Synch + CIDR vs. 5-d Pre-Synch heifers were pregnant to TAI (52.0 vs. 33.5 ± 7.3%, respectively). No difference (P = 0.33) was observed for ovulatory response after administration of GnRH on d 0 between 7-d CO-Synch + CIDR (48.2 ± 0.32%) and 5-d Pre-Synch + 7 d CO-Synch + CIDR (54.6 ± 0.31%) heifers. Using a 7-d CIDR based pre-synchronization protocol did not increase synchrony of a new follicular wave or ovulation after GnRH administration on d 0, and decreased pregnancy rates to TAI.

Key Words:Beef heifers, Estrus synchronization, Follicular waves, Pre-Synchronization, Timed AI

Introduction

Developing an estrus synchronization protocol that increases synchrony of follicular waves and pregnancy rate following a fixed-TAI is needed. Establishment of pregnancy is related to ovulation to the first injection of GnRH in an estrus synchronization protocol (Vasconcelos et al., 2001). Also, follicular turnover is dependent on the stage of follicular development at the time exogenous GnRH is administered (Geary et al., 2000; Atkins et al. 2008). By administering GnRH at random stages of the estrous cycle 45% to 60% of heifers will ovulate, and have recruitment of a new follicular wave (Moreira et al., 2000; Atkins et al., 2008). Low pregnancy rates from TAI may result from small immature dominant follicles at the time of a GnRH-induced ovulation (Atkins et al., 2008). Pre-synchronization prior to administration of an estrus synchronization protocol that uses GnRH and PG may assist in improving synchrony of follicular waves in heifers (Moreira et al., 2000; Busch et al., 2007). In addition, Atkins et al. (2005) explained that ovulatory response to GnRH is dependent on the day of the estrous cycle in which it is administered, and found the greatest ovulatory response following GnRH was on d 5 of the estrous cycle. Our hypothesis was that a 7-d controlled internal drug release (**CIDR**) based pre-synchronization protocol would increase pregnancy rates following fixed-time AI, and these females would exhibit a greater ovulatory response after administration of GnRH analogue at the beginning of CO-Synch + CIDR. The objectives of this study were to evaluate: 1) the effect of using a 7-d controlled internal drug release (**CIDR**) based pre-synchronization protocol on pregnancy rates following fixed-time AI, and 2) ovulation and follicular dynamics after administration of GnRH at the start of CO-Synch + CIDR.

Materials and Methods

Animals Six-hundred-nine beef heifers were used at 3 locations (location 1, n = 25; location 2, n = 110; location 3, n = 474). Heifers were randomly assigned within each location to 1 of 2 treatments: 1) 5-d Pre-Synch + 7-d CO-Synch + CIDR, or 2) 7-d CO-Synch + CIDR in a pairwise comparison arrangement. All injections were given intramuscularly at 100 μg of GnRH analogue and 25 mg prostaglandinF2α (**PGF**). On d –14, heifers in the 5-d Pre-Synch group were administered GnRH and CIDR. The CIDR was removed on d -7 and heifers received an injection of PGF. On d 0, all heifers received a CIDR and GnRH. The CIDR was removed and heifers received PGF on d 7. All heifers received TAI and GnRH 54 ±2 h after PGF (Figure 1).

Heifers were exposed to bulls for natural service 10 d after TAI until the end of the breeding season which varied in length by location, but averaged 45 d. Pregnancy was determined through ultrasonography at all 3 locations using a 5.0-MHz sector array transducer 50 ± 5 d after TAI. Rectal palpation was used to determine final pregnancy on d 120 ± 20. Pregnancy loss was defined as a heifer having a viable fetus at the first ultrasound, with no viable fetus at the final pregnancy diagnosis. However, females who exhibited a viable fetus at d 50 ± 5 were considered to have conceived to TAI for this study.

Ovarian Transrectal Ultrasonography Heifers at 2 locations (location 1, n=25; location 2, n=110) were used as a subset to examine ovarian structures. Transrectal ultrasonography was performed using a 5.0-MHz sector array transducer in order to determine response of ovarian structures following administration of exogenous GnRH analogue on d 0 of synchronization. On d 3 of treatment, all heifers were ultrasounded again in order to analyze response of GnRH analogue on the ovarian structures. The diameter of each follicle was measured on both ovaries, and the number of follicles from each ovary was recorded, along with the diameter and number of corpus lutea. Follicles were then classified according to their diameter: Class I (2 to 5 mm), Class II (6 to 9 mm), or Class III (>9mm) (Moreira et al., 2000, Atkins et al., 2007). A dominant follicle was defined as at least 2 mm greater than other follicles (Sirois and Fortune, 1990, Moreira et al., 2000).

Statistical Analysis The GLIMMIX procedure in SAS (SAS Inst. Inc., Cary, NC) was used to analyze differences in TAI pregnancy rate. Available factors included BCS, sire, AI technician, location, and treatment. Factors were defined significant at $P < 0.05$, while any trends were defined at $P \leq 0.10$. Factors that were included in the final model were location, treatment, and treatment X location interaction. The LOGIT procedure in SAS was used to analyze ovarian response and follicle sizes. Available factors included size of dominant follicle on d 0 and d 3, ovulatory response following GnRH, treatment, location, and treatment X location, and all were included within the final model.

Discussion

Body condition scores were collected at all locations. No difference ($P > 0.05$) was found for BCS between locations (Table 1). For 60 d prior to breeding, females at locations 2 and 3 received only pasture forage, heifers at location 1 received a high quality harvested forage diet. This may explain the lack of variation in BCS among locations.

Overall pregnancy rates at location 2 were significantly higher ($P = 0.0001$; Table 2) than the pregnancy rates at locations 1 and 3. No treatment x location interaction ($P = 0.45$) was observed for pregnancy to TAI, therefore these data were pooled across locations. At location 1, there was a tendency ($P = 0.08$) between treatments. These results were similar at location 3 where the 7-d CO-Synch + CIDR control treatment group significantly outperformed ($P = 0.003$) the 5 d pre-synch + CIDR females. At Location 2 however, both of the treatment groups performed the same, and no difference ($P = 0.45$) was found. It is inexplicable why the 5 d pre-synch + CIDR performed well at location 2, and low at both locations 1 and 3. Although, Small et al. (2009) pre-synchronized females by utilizing a CIDR for 15 d, and found that the dominant follicular size as well as ovulation rate following GnRH and removal of the CIDR was increased. Yet, pregnancy rates were not consistently improved. Dahlen et al., (2003) administered a single injection of gonadotropin releasing hormone 6 d prior to initiation of the CO-Synch protocol causing the dominant follicle to ovulate, and recruitment of a new follicular wave. This failed to improve pregnancy rates when compared to heifers that received a CO-Synch + CIDR protocol. Furthermore, Zuluaga et al., (2010) compared pregnancy rates following fixed-TAI in beef heifers that were pre-synchronized to a group that received no pre-synchronization and found that TAI pregnancy rates did not differ ($P = 0.18$) between treatments. This research also illustrated that only ovulation after administration of GnRH at the beginning of an estrous synchronization protocol was benefitted by pre-synchronization, and failed to increase fixed-TAI pregnancy rate when compared to females that were not pre-synchronized. Zuluaga et al., (2010) explained that pre-synchronization did in fact increase the proportion of females ovulating to GnRH at the initiation of an estrous synchronization protocol, but at the synchrony of new follicular wave emergence was also increased for the treatment group that was not pre-synchronized. It is also important to note that females in the current study experienced drought conditions and high climatic temperatures during the synchronization process. It is known that heat stress and elevated body temperature negatively affects fertility, ovarian function and expression of estrus consequently decreasing the likelihood of these females to become pregnant to TAI (Amundson et al., 2006). It is also tempting to hypothesize that because of the drought year, heifers were not on an increasing plane of nutrition during the synchronization process and breeding, which may have also had a negative impact on becoming pregnant to TAI.

Based upon ultrasound data collected on d 0 and d 3 of treatment, no difference ($P = 0.33$) was observed for ovulatory response and emergence of a new follicular wave after administration of GnRH between the 7-d CO-Synch + CIDR and 5-d Pre-Synch + 7 d CO-Synch + CIDR heifers. Yet, a tendency ($P = 0.07$) was found for a treatment X location interaction between locations 1 and 2. There was also a tendency ($P = 0.07$) for 5-d Pre-Synch heifers to have an increased ovulatory response following GnRH based upon the d 0 and d 3 ultrasound of ovarian structures. No difference ($P = 0.77$) was found for ovulatory response following administration of GnRH at location 2 or overall (Table 3). The results at location 1 are consistent with Moreira et al., (2000) stating that synchrony of the emergence of a new follicular wave following administration of GnRH was improved on d 5 of the estrous cycle. Along with Atkins et al., (2008), which also explained that the GnRH induced LH surge had the greatest efficacy on d 18 followed by d 5, 15, 10, and 2. Perhaps there may have been a lack of ability to detect any differences at location 1 and not location 2 due to the increase sample size at location 2.

Implications

Although synchrony of an emergence of a new follicular wave was seen at one location, the 5-d Pre-Synch treatment group failed to improve overall pregnancy rates at all three locations. Further research must be conducted analyzing the optimal day to initiate treatment that will increase synchrony as well as pregnancy rates following a fixed- TAI.

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Table 1. BCS as well as overall pregnancy rate following fixed-TAI of beef heifers by location

Location	n =	BCS[1]	Overall percent pregnant to TAI
1	25	5.5 ± 0.70	29.87[a]
2	110	4.8 ± 0.60	55.59[b]
3	474	5.1 ± 0.52	39.83[a]

[1] Body Condition scores were collected and analyzed using the 9-point scale (1 = thin, 9 = obese; Richards et al., 1986)
[ab] means within a column, lacking a common superscript differ ($P < 0.05$)

Table 2. Pregnancy rates to fixed-TAI in beef heifers by estrous synchronization treatment by location as well as overall

	Treatment			
	5-d Pre-Synch[1]	7-d CO-Synch + CIDR[2]		
Location	%	%	SEM[3]	*P* - Value
1	15.38	50.00	19.5	0.08
2	51.85	59.26	4.5	0.43
3	33.19	46.88	9.3	0.003
Overall[4]	33.5	52.0	7.3	0.01

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) on d -14, and 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im, d – 7 CIDR removed and 25 mg PGF (Lutalyse, Pfizer Animal Health)im. On d 0 heifers received a CIDR and 100 µg GnRH analogue, d 7 CIDR removed and PGF 25 mg. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[2]Heifers received a CIDR and 100 µg GnRH analogue i.m. on d 0, on d 7 the CIDR was removed and 25 mg PGF. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[3]Standard Error of the Mean
[4] No treatment x location interaction ($P = 0.45$) was observed for pregnancy to TAI, therefore these data were pooled across locations.

Table 3. Ovulatory response to exogenous GnRH for a d 5 pre-synchronized estrous cycle vs. no pre-synchronization

	Treatment			
	5-d Pre-Synch	7-d CO-Synch + CIDR[2]		
Location	%[4]	%	SEM[3]	*P* - Value
1	92.31 (12/13)	58.33 (7/12)	1.19	0.07
2	70.37 (38/54)	67.86 (38/56)	0.41	0.77

[1] Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) on d -14, and 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im, d – 7 CIDR removed and 25 mg PGF (Lutalyse, Pfizer Animal Health)im. On d 0 heifers received a CIDR and 100 µg GnRH analogue, d 7 CIDR removed and PGF 25 mg. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[2]Heifers received a CIDR and 100 µg GnRH analogue i.m. on d 0, on d 7 the CIDR was removed and 25 mg PGF. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.
[3]Standard Error of the Mean
[4]Percent of females who ovulated following exogenous GnRH analogue. Heifers were ultrasounded on d 0 of treatment and sizes of all follicles and corpus lutea were recorded. Heifers were ultrasounded again on d 3 of treatment, and each female was considered to have ovulated if a reduction of the dominant follicle was seen.

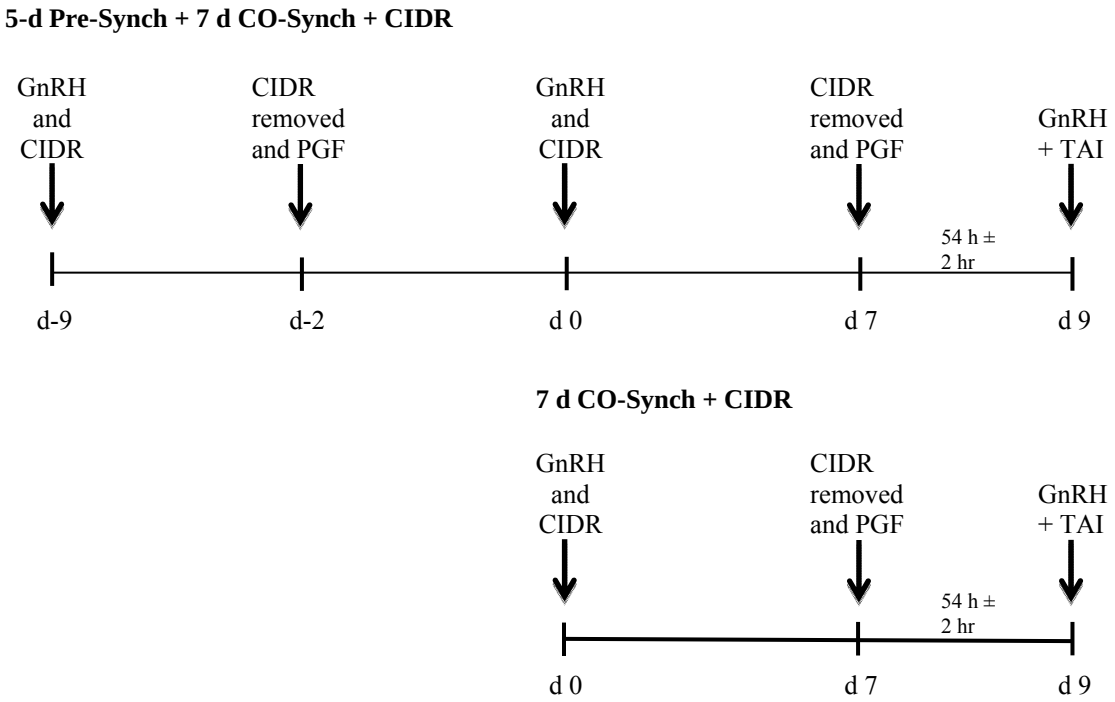

Figure 1. Illustration of the 5-d Pre-Synch + 7 d CO-Synch + CIDR and 7 d CO-Synch + CIDR protocols that heifers received. Heifers received a CIDR (Pfizer Animal Health, New York, NY; 1.38 g of progesterone) on d -14, and 100 µg GnRH analogue (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) im, d – 7 CIDR removed and 25 mg PGF (Lutalyse, Pfizer Animal Health)im. On d 0 heifers received a CIDR and 100 µg GnRH analogue, d 7 CIDR removed and PGF 25 mg. Heifers received TAI and 100 µg GnRH analogue 54 ±2 h following PGF.

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EFFECTIVENESS OF A DRIED MOLASSES LICK TUB SUPPLEMENT CONTAINING CALCIUM SALTS OF FISH OIL FOR INCREASING OMEGA-3 FATTY ACIDS IN MUSCLE OF FORAGE-FINISHED CATTLE

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ABSTRACT: Supplementing *n*-3 fatty acids to grazing cattle requires a low labor input delivery system to be of use for producers. Our previous work indicated that intake of calcium salts of fish oil (**FO**) was strongly correlated with concentrations of eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) in *Longissimus dorsi* (**LD**) of forage-finished cattle, but that intake was highly variable when fed as a dry mixture. Our hypothesis was that calcium salts of FO fatty acids provided as a dried molasses lick tub supplement will result in greater concentrations, as well as minimal variation in concentrations of *n*-3 fatty acids in LD of forage-finished cattle. The objective was to compare concentrations of LD alpha-linolenic acid (**ALA**), EPA, and DHA in forage-finished cattle supplemented with a dried molasses lick tub supplement that contained 30% of either the calcium salt of FO or of palm oil (**PO**). Fourteen heifers and 14 steers (Angus, initial body weight 235.3 ± 0.2 kg and 267.3 ± 3.3 kg, respectively) were allotted randomly by weight so that a 2 x 2 factorial experiment composed of seven steers and heifers each were represented in the two dietary supplement treatments. Within each treatment cattle were group fed so that variation in LD ALA, EPA, and DHA could be determined when a producer-oriented, group grazing system was employed. Cattle were provided free choice harvested forage composed of 65% hay and 35% haylage mixture (both forages were 50% alfalfa; 25% wheat grass; 25% brome-grass; CP = 15.2%) in dry lot. Dried molasses lick tubs (114 kg) were offered continuously for 220 d. Cattle were harvested and LD sampled 14 d postmortem. Fatty acid composition was determined by GLC. Concentrations (mg/100 g fresh LD) of ALA, EPA, and DHA were greater ($P \leq 0.05$) in LD of cattle supplemented with FO than with PO. Steer LD contained greater ($P = 0.05$) concentrations of EPA than heifers. For LD concentrations of EPA, the major fatty acid of the FO supplement, coefficient of variation (38.1%) was 28% less with the dried molasses lick tubs than was observed in our previous work supplementing the FO as a dry mixture. We conclude that the dried molasses lick tub delivery system for calcium salts of FO is effective for increasing *n*-3 fatty acids in LD of forage-finished steers and heifers.

Key Words: Bovine; Fatty acid composition; Heifers; Longissimus dorsi; Steers.

Introduction

Consumer diet and nutrient awareness has led to rising demand for a leaner, more nutritious meat product. Forage-fed cattle offer a lean meat option with a fatty acid profile more reflective of the forage consumed, especially with increased concentrations of *n*-3 fatty acids. Although concentrations of *n*-3 fatty acids are higher in meat of forage-fed beef (Rule et al., 2002; Nuernberg et al., 2005) previously documented literature does not support the assertion that forage-fed beef is a comparatively rich source of *n*-3 fatty acids. Attempts to increase tissue concentrations of eicosapentaenoic acid (**EPA**, 20:5 *n*-3) and docosahexaenoic acid (**DHA**, 22:6 *n*-3) have shown mixed results, largely due to ruminal biohydrogenation. Using a by-pass source of EPA and DHA could increase tissue concentrations of these fatty acids; however, the extent to which supplemental *n*-3 fatty acids can be increased in tissues of forage-fed beef has not been reported. Calcium salts of fish oil (**FO**) fatty acids were shown to provide limited ruminal by-pass, which resulted in increased concentrations of EPA and DHA in *M. Longissimus dorsi* (**LD**; 12th rib) and other tissues, including blood serum of cattle grown on irrigated pasture (Rule et al., 2011). The FO calcium salts were individually fed as a mixture with ground, dried beet pulp as a carrier; the fishy odor was

prevalent, and intake was quite variable. However, LD and serum EPA and DHA concentrations were correlated strongly (r = 0.67; our unpublished data) with intake. Although LD concentration of EPA and DHA were increased compared with palm oil (**PO**) calcium salts, variation in intake and delivery of the FO supplement would require improvement before such a strategy could be implemented at the producer level. Dried molasses lick tubs (**lick tubs**) are currently used for delivery of dietary mineral and protein supplements for beef cattle. In a preliminary study we fed 114-kg lick tubs that contained 30% by weight calcium salts of FO fatty acids to mature Angus cows maintained on late-fall native grass pasture. Serum EPA and DHA were increased indicating that the lick tub delivery system could be a viable means of supplementing the FO calcium salts. Furthermore, no fishy odor was obvious and all cows were observed to consume the lick tubs, indicating greater palatability which could decrease variation in intake resulting in more uniform increases in tissue EPA and DHA. Our hypothesis was that calcium salts of FO fatty acids provided as a dried molasses lick tub supplement will result in greater concentrations, as well as minimal variation in concentrations of *n*-3 fatty acids in LD of forage-finished cattle when compared to a PO control. The objective of this study was to determine concentrations and variation in LD alpha-linolenic acid (**ALA**), EPA, and DHA in forage-finished cattle supplemented with a lick tub that contained 30% calcium salt of either FO or PO.

Materials and Methods

The University of Wyoming Institutional Animal Care and Use Committee approved all procedures for the following study. Fourteen heifers and 14 steers (Angus, initial body weight 235.3 ± 0.2 kg and 267.3 ± 3.3 kg, respectively) were randomly allotted by weight into either a FO-based fatty acid calcium salt or a PO-based fatty acid calcium salt treatment so that seven steers and heifers each were represented in the two dietary supplement treatments. Calcium salts of fatty acids were provided by Virtus Nutrition (Corcoran, CA) and lick tubs that contained 30% by weight of fatty acid calcium salts were prepared by Ridley Block (Whitewood, SD). The original objective was for grazing; however, due to lack of re-growth caused by severe drought in eastern Wyoming, all cattle were raised in dry lot. To mimic the group feeding environment of pasture grazing the 14 cattle on each lick tub treatment were raised in a single dry lot pen for the two supplements. This method also allowed for determination of variation in LD concentrations of ALA, EPA, and DHA when a producer-oriented, group grazing system was employed. Two 114-kg lick tubs were placed in each pen until 95% of the supplement was consumed and then replaced with a full lick tub, which were available continuously for 220 d. Cattle were provided free-choice harvested forage composed of 65% hay and 35% haylage (50% alfalfa, 25% brome-grass, and 25% wheat grass mixed forage). Cattle were weighed every 45 d, and harvested after 220 d at the University of Wyoming Meats Laboratory; LD were sampled 14 d postmortem. At each weighing, forage samples from each bunk and lick tub samples were obtained and composited. Muscle fatty acid methyl esters were prepared by direct transesterification with 0.2 *N* KOH in methanol (Murrieta et al., 2003). Forage and supplement fatty acid methyl esters were prepared using methanolic-HCl (Weston et al., 2008). Fatty acid concentrations were determined using GLC; reaction tubes contained 1.0 mg of C13:0; whereas, feed samples contained 1.0 mg of C21:0 as internal standard.

Fatty acid data were analyzed as a 2 x 2 factorial designed experiment using the GLM procedure of SAS to determine effects of supplement and sex on LD fatty acids and to calculate least squared means.

Results and Discussion

Forage contained: CP, 15.2%; ADF, 49.1%; NDF, 53.9%. Fatty acid concentrations (mg/g DM) of the harvested forage were: 16:0, 2.6 (21.1% of total fatty acids); 18:0, 0.4 (3.3%); 18:1 *n*-9, 0.4 (3.3%); 18:2 *n*-6, 1.8 (14.6%); 18:3 *n*-3, 2.2 (17.9%); total fatty acids, 12.3. Pen average intake of FO lick tub was 0.22 kg/d and 0.23 kg/d of the PO lick tub.

Table 1. Concentration (mg/g of lick tub) of ALA, EPA, and DHA in palm oil and fish oil lick tubs

Fatty acid	Palm oil	Fish oil
ALA	10.2	14.0
EPA	-	182.4
DHA	-	52.4

Concentrations of ALA were comparable between PO and FO supplements (Table 1). Concentrations of EPA and DHA were reflective of the FO in the supplement; whereas, these *n*-3 fatty acids do not occur in plant lipids.

Initial and final body weights and ADG were similar (P > 0.05) for each supplement treatment (Table 2). However, heifers were lighter ($P < 0.01$) initially and at 220 d than steers, but without a sex effect on ADG ($P = 0.30$).

Table 2. Body weights and ADG of heifers and steers fed calcium salts of fish oil (FO) or of palm oil (PO)

Supplement effect				
Item	FO	PO	SEM	*P* value
Initial BW[a]	249.73	252.8	7.1	0.76
Final BW[a]	410.0	428.5	10.3	0.21
ADG, 220 d	0.73	0.80	0.03	0.10
Sex effect				
Item	Heifer	Steer	SEM	*P* value
Initial BW[a]	235.3	267.3	7.1	< 0.01
Final BW[a]	398.4	440.2	10.3	< 0.01
ADG, 220 d	0.74	0.79	0.03	0.30

[a] Kg.

Effects of lick tub treatment and of sex on concentrations (mg /100 g of fresh LD) of fatty acids in LD are shown in Table 3. Concentration of 18:1 *trans*-11 was greater ($P < 0.01$) for FO than PO supplemented cattle; however, this was not accompanied by a difference in CLA ($P = 0.15$). Concentrations of 18:2 *n*-6, 20:3 *n*-6, and 20:4 *n*-6 were greater ($P < 0.01$) for the PO than FO supplemented cattle. Concentrations of 18:3 n-3, 20:0, EPA, and DHA were greater ($P < 0.03$) for FO than for PO supplemented cattle. Overall, concentrations of the long-chain *n*-3 fatty acid derived from the FO supplement were increased in these cattle compared with the PO supplemented cattle. Differences in EPA and DHA between the FO and PO treatments in the present study were comparable to differences observed previously (Rule et al., 2011). The only sex effect on LD fatty acids observed was for concentration of EPA, which was greater ($P = 0.05$) for steers than heifers; the reason for this difference is not understood.

A major goal of the present study was to compare variation in LD concentrations of the long-chain *n*-3 fatty acid, EPA, with variation in this fatty acid in LD observed in our previous study (Rule et al., 2011). In the 2011 study cattle grazed irrigated pasture and were individually fed supplements that contained either calcium salts of FO or PO that were prepared as a dry mixture with dried, ground beet pulp. Mean LD concentration of EPA of the PO fed cattle was subtracted from each of the values for the 12 cattle fed the FO supplement to calculate the change in EPA due to the FO supplement. The same calculation was made for LD EPA concentrations of the current lick tub study so that variation in EPA between the two studies could be contrasted. From the 2011 study, LD EPA concentration (mg/100g of fresh LD) mean, standard deviation, range, and coefficient of variation were: 8.57, 4.53, 1.55 to 14.81, and 52.82%, respectively. From the current lick tub study, the same calculations resulted in 7.90, 3.01, 3.83 to 15.38, and 38.09%, respectively. Mean concentrations of EPA were comparable for the two studies, but standard deviation and coefficient of variation were less for the lick tub delivery of FO calcium salts. The upper end of the range of concentrations were comparable, but the lower end was less for the previous grazing study indicating that the reduced variation observed in the lick tub study was likely from more cattle readily consuming the supplement when delivered as the dried molasses product. However, cattle were provided tubs while housed in pens as opposed to a larger area typical of pastures, which could also influence the outcome. Wijendran and Hayes (2004) suggested a daily intake of at least 0.5 g of EPA plus DHA by people for optimal cardiovascular health. The concentrations of these fatty acids in the LD of the current study would provide about 36 mg of EPA + DHA in a 227 g steak, which would contribute about 7% of the suggested daily intake. Further work will be necessary to determine the effect of forage quality and of supplementation during pasture feeding. We conclude that the dried molasses lick tub delivery system is effective for increasing *n*-3 fatty acids in LD of forage-finished steers and heifers.

Implications

Finishing growing steers and heifers on forage along with a supplement containing calcium salts of fish oil fatty acids using the dried molasses lick tub delivery system will effectively increase muscle concentrations of α - linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid.

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Table 3. Concentrations of fatty acids in LD (mg/100 g of fresh LD) of forage-finished heifers and steers supplemented with dried molasses lick tubs that contained either calcium salts of palm oil or fish oil

	Supplement		Sex			*P*-value	
Fatty Acid[a]	Palm oil	Fish oil	Heifer	Steer	SEM[b]	Supplement	Sex
14:0	32.2	29.8	32.0	29.9	3.3	0.61	0.65
14:1	4.5	3.8	4.4	3.9	0.6	0.38	0.51
15:0	6.7	7.0	6.7	7.0	0.7	0.75	0.80
15:1	2.6	2.6	2.3	2.8	0.5	0.93	0.47
16:0	349.3	306.0	340.2	315.1	30.2	0.32	0.56
16:1	22.5	25.7	25.0	23.1	2.7	0.40	0.63
17:0	13.9	15.2	14.6	14.6	1.4	0.53	0.99
17:1	6.3	5.4	5.8	5.9	0.6	0.31	0.96
18:0	180.4	171.6	176.7	175.3	16.0	0.70	0.95
18:1 *trans*-11	3.4	5.8	4.4	4.8	0.5	< 0.01	0.53
18:1 *cis*-9	341.8	274.6	324.8	291.7	25.1	0.07	0.36
18:1 *cis*-10	12.6	14.4	13.7	13.3	1.1	0.25	0.81
18:2 *n*-6	44.5	32.9	39.4	37.9	2.5	< 0.01	0.67
18:3 *n*-3	10.7	13.3	12.0	12.1	0.9	0.05	0.93
CLA[c]	2.9	2.1	2.6	2.4	0.4	0.15	0.75
20:0	1.4	2.5	1.8	2.2	0.4	0.03	0.41
20:3 *n*-6	4.9	2.7	3.9	3.8	0.3	< 0.01	0.87
20:4 *n*-6	14.4	9.5	11.4	12.4	0.7	< 0.01	0.29
EPA[c]	4.7	12.6	7.8	9.4	0.6	< 0.01	0.05
DHA[c]	0.4	3.1	1.4	2.1	0.3	< 0.01	0.09

[a]Fatty acids denoted as number of carbon atoms : number of carbon-carbon double bonds.
[b]Standard error of the least squared mean; n = 14.
[C]18:2 $^{\Delta}$9-*cis*, 11-*trans*; EPA = 20:5 *n*-3; DHA = 22:5 *n*-3.

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IMPACTS OF SUPPLEMENTAL ARGININE ON REPRODUCTIVE PERFORMANCE IN SHEEP[1]

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ABSTRACT: In the U.S. sheep industry embryonic and fetal death during pregnancy can account for 25 to 50% of the total number of ovulations. Research from this laboratory has shown supplemental arginine (Arg) can recover embryonic and/or fetal loss in fall lambing ewes synchronized to estrus with exogenous hormones. The objective of this study was to determine the effects of injectable (Exp. 1) and oral (Exp. 2) Arg supplementation provided 2 wk post breeding on reproductive performance of naturally stimulated fall lambing ewes. Rambouillet ewes were exposed to intact rams equipped with marking harnesses to induce cyclicity. Upon estrus detection (d 0), ewes were randomly assigned to one of six treatments for a 14 d treatment period: IV-saline **(CON**; n = 25), IV-alanine (**IVALA**; n = 20), IV-arginine (**IVARG**; n = 23), rumen-protected Arg (**RPARG**; n = 20), corn and soybean meal (**SBM**; n = 23), or corn and fishmeal (**FM**; n = 24). Daily treatments, except CON, IVALA, and SBM, were formulated to provide supplemental Arg at approximately 30 mg/kg ewe BW. Ewes receiving IV treatments were provided 454 g/d corn post-injection. Oral supplements were ground and provided individually to ewes at 0800 daily. Blood samples were collected on d 0, 2, 4, 6, 8, 10, 12, and 14 from 12 ewes per treatment to evaluate serum progesterone concentrations. At lambing, birth weight, birth type, and sex were recorded. Weaning weights were recorded when the average age of lambs was 85 d. No differences ($P \geq 0.35$) were detected for pregnancy and lambing rates or birth and weaning weights among treatments for either experiment. No differences ($P \geq 0.14$) were detected for progesterone concentration for treatment or treatment × day interactions in Exp. 1 or 2. In contrast to previous research, supplemental Arg during the first 14 d of pregnancy did not improve ewe reproductive performance.

Key Words: arginine, progesterone, reproduction, sheep

Introduction

Reproductive loss accounts one of the largest economic inefficiencies in any livestock operation, although often unnoticed. The majority of embryonic loss occurs before d 18 of gestation (Hulet et al., 1956; Moore et al., 1960; Quinlivan, 1966). Loss of individual embryos can occur without a complete loss of pregnancy, such as in the case of multiple fetuses (Rhind et al., 1980; Schrick and Inskeep, 1993). In sheep, it has been reported that 30% of fertilized ova are not represented by live births, resulting in frequent, but unrecognized economic loss (Bolet, 1986; Knights et al., 2003; Dixon et al., 2007). Strategies to enhance prenatal growth and survival could clearly have a major economic impact in the sheep industry. Past research at NDSU has shown supplemental arginine (Arg) can improve reproductive success in fall lambing ewes synchronized to estrus with exogenous hormones (Luther et al., 2009; Saevre et al., 2011).

The amino acid L-arginine is a precursor for nitric oxide and important in the synthesis of polyamines and proteins, all of which are essential to proper development of the embryo and placenta. Gestating sows supplemented with Arg achieved a 22% increase in live piglets born when compared with non-supplemented sows (11.4 vs. 9.4, respectively; Mateo et al., 2007). Treatment of ewes with injectable L-Arg during maternal recognition of pregnancy improved pregnancy rate by 24% (Saevre et al., 2011). Similarly, supplementation of Arg (injectable) for 15 d post-breeding increased lambing rate by 50% (Luther et al., 2009). Previous research used an injectable Arg source, which is not feasible for commercial livestock operations. In order for this supplement to become more commercially applicable an oral form of supplemental Arg should be developed. The objective of this study was to determine the effects of injectable (Exp. 1) and oral (Exp. 2) Arg supplementation provided 2 wk post-breeding on reproductive performance of naturally stimulated fall lambing ewes. Our hypothesis was that Arg would increase pregnancy and lambing rates while decreasing postnatal lamb loss.

Materials and Methods

All procedures were approved by the Animal Care and Use Committee of North Dakota State University. This study was conducted at the Hettinger Research Extension Center in Hettinger, ND.

Animals and Diets

In April of 2012, 210 multiparous Rambouillet ewes (64.7 ± 6.8 kg BW) were supplemented with 454 g of corn

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and exposed to 15 ram lambs to induce estrus. Ram lambs equipped with marking harnesses were introduced to ewes d -12 prior to the initiation of the study. Mature Rambouillet rams (n = 10) equipped with marking harnesses were introduced to the ewe flock on d 0. Ewes that were marked by ram lambs prior to study initiation were removed from the trial. Ewes were monitored daily at 0800 to determine onset of estrus as indicated by breeding marks from harnessed mature rams. Once estrus was detected, ewes were randomly assigned to one of six treatment groups: control (**CON**; n = 25), IV-alanine (**IVALA**; n = 20; Ajinomoto North America, Inc., Raleigh, NC), IV-arginine (**IVARG**; n = 23; Ajinomoto North America, Inc., Raleigh, NC), rumen-protected arginine (**RPARG**; n = 20), soybean meal (**SBM**; n = 23), and fishmeal (**FM**; n = 24). Treatment groups were separated into injectable and oral groups (Exp. 1 and 2, respectively). In Exp. 1, all ewes received 454 g of corn daily and were injected with similar treatment volumes (0.100 mL • kg BW^{-1} • d^{-1}). Intravenous injections of Arg (30 mg • kg BW^{-1} • d^{-1} of l-arginine), alanine (Ala; 30 mg • kg BW^{-1} • d^{-1} of l-alanine), and saline were administered daily to IVARG, IVALA, and CON ewes, respectively. Similar to Luther et al. (2009) and Saevre (2011), the IVARG and IVALA treatments were designed to provide 30 mg • kg BW^{-1} • d^{-1} of metabolically available Arg or Ala, respectively. In Exp. 2, all ewes received 454 g/d of their respective treatments. Treatments were: RPARG (0.15 g/kg BW of rumen protected product mixed with ground corn), SBM (25:75 soybean meal:corn), and FM (37.5:62.5 fishmeal:corn). The RPARG and FM treatments were designed to provide 30 mg • kg BW^{-1} • d^{-1} of metabolically available Arg,, whereas SBM was designed to be isocaloric to FM. The CON treatment from Exp. 1 served as the CON treatment for Exp. 2. During the entire treatment period, ewes were group fed a basal ration of 1.88 kg of alfalfa hay and 0.226 kg of barley haylage (DM basis; Table 1). From d 0 (estrus) until d 14, ewes individually received their assigned treatment. All treatments were imposed within 15 d of mature ram introduction.

Sample Collection

Blood samples were collected via jugular venipuncture into 10 mL serum tubes (BD Vacutainer Serum, Becton, Dickinson and Company, Franklin Lakes, NJ) on d 0, 2, 4, 6, 8, 10, 12, and 14 from 12 ewes per treatment prior to administration of treatment at 0800 and immediately placed on ice. Samples were then centrifuged at 4° C for 30 min at 1500 × g and serum was transferred into plastic 2.0 mL mirocentrifuge tubes and frozen at -20° C until assayed. Blood samples were assayed for concentrations of circulating progesterone using IMMULITE 1000 (Siemens Healthcare Diagnostics; Galbreath et al., 2008). At lambing, birth weight, birth type, and sex were collected. Weaning weights were collected when the average lamb age was 85 d.

Statistical Analyses

Pregnancy, prolificacy, and lambing rates were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). Birth BW, weaning BW, and progesterone concentrations were analyzed using the MIXED procedure of SAS. Dietary or IV treatment served as the fixed effect. The fixed effect of day was utilized in the REPEATED measures analysis for progesterone concentrations. The model included fixed effects of dietary treatment, day, and treatment × day. Birth rate and sex of lamb were added into the model as random effects for birth and weaning BW. Significance was determined at $P \leq 0.05$. To partition day effects and treatment × day interactions, LS Means were utilized ($P \leq 0.05$).

Results

In Exp. 1 no differences ($P \geq 0.35$; Table 2) were detected for pregnancy, prolificacy, or lambing rates among treatments due to IV treatment. Similarly, in Exp. 2 no differences ($P \geq 0.61$; Table 3) were detected for pregnancy, prolificacy, or lambing rates among treatments due to dietary treatment. Additionally, for progesterone concentrations there were no differences ($P \geq 0.14$) detected for treatment or treatment × day interactions among treatments in Exp. 1 or 2 (Figures 1 and 2, respectively); however, there was a day effect ($P < 0.0001$) for both Exp. 1 and 2, with progesterone concentrations increasing from d 1 through d 14 ($P \leq 0.05$). Similar to prenatal data, no differences among treatments ($P \geq 0.53$) were detected for birth BW or weaning BW in Exp. 1 and 2 (Tables 2 and 3, respectively).

Discussion

Arg is important for many biological functions, including synthesis of nitric oxide (Gouge et al., 1998; Manser et al., 2004). Other researchers have hypothesized that treating ewes with Arg at, or slightly before, the time of maternal recognition of pregnancy may enhance the survival of the embryo during early embryogenesis (Luther et al., 2009). This is likely accomplished through the role Arg plays in polyamine and nitric oxide synthesis.

In the present study, pregnancy rates were not influenced through injectable or oral treatments. In contrast, research from this laboratory reported greater pregnancy rates in ewes supplemented with injectable Arg from d 9 through 14 post-breeding (Saevre et al., 2011). However, Luther et al., (2009) observed no differences in pregnancy rates for supplementation with injectable Arg from d 0 through 14. In the previous studies (Luther et al., 2009; Saevre et al., 2011), pregnancy rates were 55% for ARG vs. 45% for CON, and in the present study were 88% for IVARG vs. 88% for CON vs. 86% RPARG. The differences in pregnancy rates between these studies could be due to a difference in estrus synchronization techniques utilized. Ewes in the previous two studies were synchronized artificially with a controlled internal drug release inserts and an injection of PG600 (containing 400 IU of equine chorionic gonadotropin and 200 IU of human chorionic gonadotropin), whereas the ewes in the present study were naturally synchronized using ram exposure. In the present study, only 1.5% of ewes displayed estrus prior to the start of the trial, indicating that most of the flock was in a period of anestrus before ram introduction.

Prolificacy was also not influenced through injectable or oral treatments in the present study. Similarly, Saevre et al., (2011) showed no influence on prolificacy through injectable L-Arg. However, Luther et al., (2009) demonstrate differences in prolificacy with 1.6 lambs born per ewe for L-Arg treated ewes vs. 1.1 for the control ewes. In the current study, we observed a lambing rate of 1.2 lambs born per ewe. Ovulation rates in out-of-season ewes are expected to decrease when compared with in-season ewes. Lunstra and Christenson (1981) demonstrated with administration of progesterone and PMSG, ovulation rates of out-of season ewes could be increased by 50%. Due to these varying results, more research is needed on the use of exogenous hormones in anestrous ewes and the effects on lambing rates for in-season and out-of-season ewes.

Therefore, in the current study utilizing ewes synchronized for estrous through ram exposure for fall lambing, we reject the hypothesis that supplemental Arg will increase pregnancy rates and lambing rates while decreasing postnatal lamb loss.

Implications

Although some research suggests that embryonic survival in sheep and other livestock operations can be enhanced when ewes are supplemented with Arg, we did not detect any improvements in reproductive performance or lamb growth in ewes supplemented with either injectable, non-rumen-protected, or rumen-protected forms of Arg. We feel further research is warranted to determine rate of reproductive loss from different methods of nonseasonal estrus induction and the ability of supplemental Arg to recover these losses.

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Table 1. Composition of basal ration[1]

	Diet Ingredients and Nutrient Concentrations	
Item	Alfalfa hay	Barley haylage
Composition	92.01	33.12
CP, % DM	18.1	12.4
ADF, % DM	43.2	31.6
TDN, % DM	53.3	66.5
Ca, %DM	1.75	0.39
P, % DM	0.22	0.38
Cu, ppm	8	6

[1]Average of 1.88 kg of alfalfa hay and 0.226 kg of barley haylage offered per ewe daily (DM basis)

Table 2. Effects of daily injection of l-arginine, l-alanine, and saline 2 wk post-breeding on reproductive performance in ewes (Exp. 1)

	Treatment[1]				
Item[3]	CON	IVALA	IVARG	SEM	*P*-value[2]
Pregnancy	88	91	88	7.1	0.95
Prolificacy	1.32	1.21	1.43	0.11	0.35
Lambing Rate	1.16	1.10	1.25	0.13	0.70
Birth BW, kg	5.4	5.4	5.2	0.24	0.57
Weaning BW, kg	24.6	22.1	22.9	1.4	0.53

[1]CON = IV saline; IVALA = 30 mg • kg BW^{-1} • d^{-1} l-alanine; IVARG = 30 mg • kg BW^{-1} • d^{-1} l-arginine

[2]*P*-value for the overall F test of treatment (n = 25, 20, and 23 for CON, IVALA, and IVARG, respectively)

[3]Pregnancy = percent pregnant per ewe treated; Prolificacy= lambs per ewe lambed; Lambing rate = lambs per ewe treated

Table 3. Effects of daily oral l-arginine supplementation 2 wk post-breeding on reproductive performance in ewes (Exp. 2)

	Treatment[1]					
Item[3]	CON	RPARG	FM	SBM	SEM	*P*-value[2]
Pregnancy	88	91	88	83	8	0.95
Prolificacy	1.32	1.17	1.17	1.26	10	0.61
Lambing Rate	1.16	1.00	1.04	1.04	0.12	0.80
Birth BW, kg	5.4	5.3	5.3	5.2	0.26	0.73
Weaning BW, kg	24.6	25.7	23.3	23.9	1.4	0.57

[1]CON = 454 g/d corn; RPARG = 454 g/d corn and 0.15 g • kg BW^{-1} • d^{-1} rumen-protected Arg; FM = 454 g/d 25:75 FM: corn; SBM = 454 g/d 37.5:62.5 SBM: corn

[2]*P*-value for the overall F test of treatment (n = 25, 20, 24, and 23 for CON, RPARG, FM, and SBM, respectively)

[3]Pregnancy = percent pregnant per ewe treated; Prolificacy= lambs per ewe lambed; Lambing rate = lambs per ewe treated

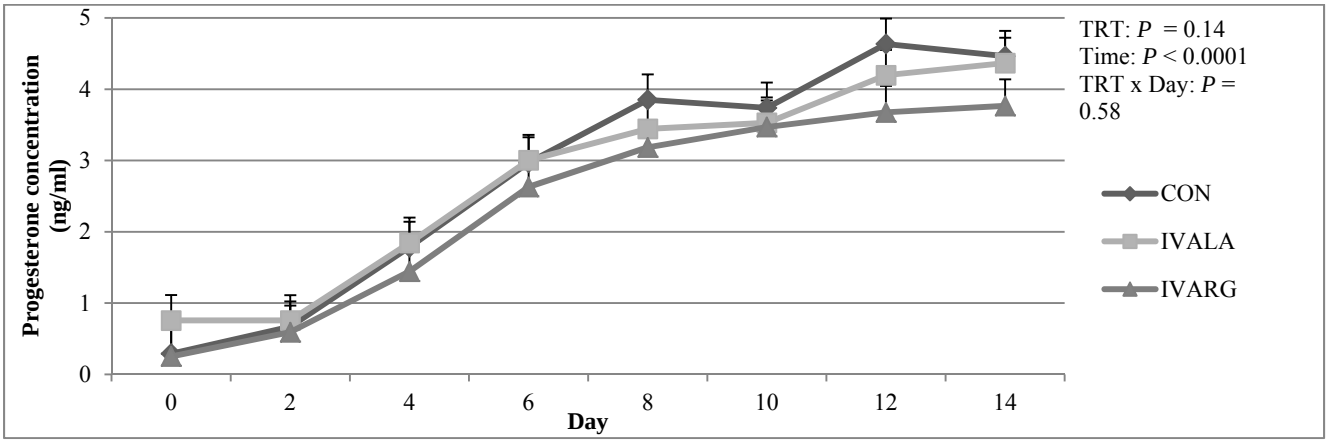

Figure 1. Effects of daily injection of saline (CON; n=25), l-alanine (IVALA; n = 20) and l-arginine (IVARG; n = 23) 2 wk post-breeding on progesterone concentrations in ewes. Data are least squared means ± SEM.

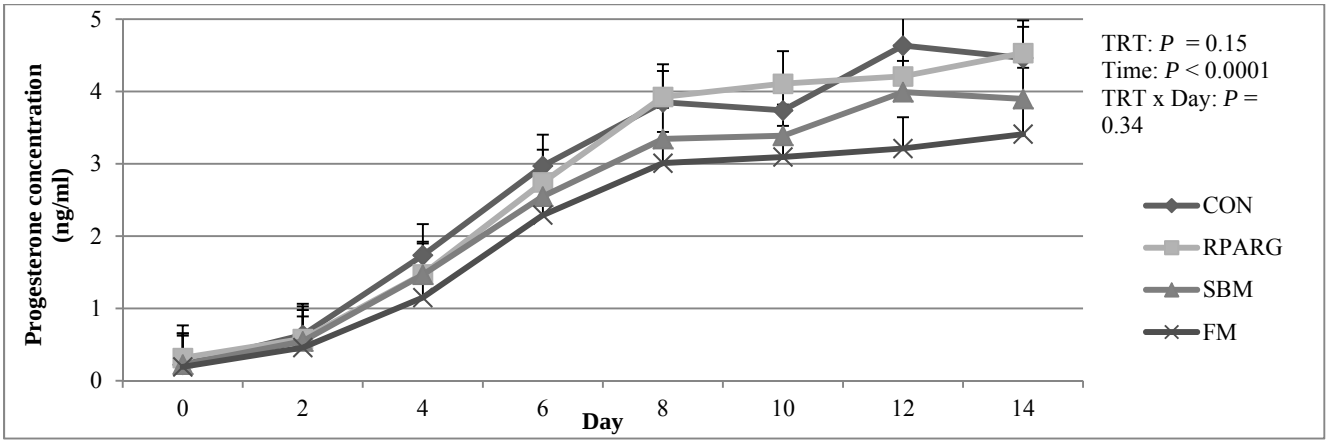

Figure 2. Effects of daily oral supplementation of 454 g of corn (CON; n = 25), Rumen-protected Arg (RPARG; n = 20), Fish meal (FM; 25:75 ration; FM: corn; n = 24); Soybean meal (SBM; 37.5:62.5 ration; SBM: corn; n = 23) 2 wk post-breeding on progesterone concentrations in ewes. Data are least squared means ± SEM.

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GENETIC EVALUATION OF THE PROBABILITY OF LAMBING IN YEARLING TARGHEE EWES

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ABSTRACT: The objective of this study was to determine the additive genetic control of lambing percentage in yearling Targhee ewes. The records of 3,103 ewe lambs born from 1989 to 2011 and mated at approximately 7.5 mo of age were analyzed. Records included sire, dam, weaning weight, breeding pen, age of dam, and lambing data. Lambing data were recorded as a binomial trait. Live or stillborn, full-term lambs were recorded as a lambing success (i.e., 1) for a yearling ewe; failure to produce a full-term lamb by a ewe was indicated in the data with a 0. The 314 sires of the ewe lambs averaged 9.6 daughters/sire; 146 sires had ≤ 5 daughters, and 36 sires had ≥ 20 daughters. The 1,770 dams of the ewe lambs averaged 1.85 daughters/ewe, with a range from 1 to 8. Yearling lambing percentage (n lambed/n mated) × 100 varied from 26 to 79% across years and averaged 49.6%. Lambing percentage of yearling ewes varied among sires from 0 to 100%, and values for sires with ≥ 20 daughters ranged from 23 to 88%. Lambing was analyzed as a threshold trait with an underlying continuous distribution. The genetic model included fixed effects of year, breeding pen, and age of dam, with adjusted weaning weight as a covariate. The relationship matrix included 6,877 animals and included a minimum of 4 generations of pedigree information, with correspondingly more generations included for later-recorded animals. The heritability estimate of lambing percentage on the underlying scale was 0.18 ± 0.04. The EBV for lambing percentage of sires of ewe lambs on the observed scale ranged from −37 to 36% and averaged -1%. The 10th and 90th percentiles of sire EBV were −20 and 17%, respectively, which indicated that sires in the 90th percentile would be expected to produce daughters with an 18.5% greater likelihood of lambing at 1 yr of age than sires with EBV in the 10th percentile.

Keywords:
sheep
fertility
genetics
lambing

Introduction

Breeding ewes to lamb at 1 yr of age has been proposed for several decades (Hume, 1939; Spencer et al., 1942; Cannon and Bath, 1969; Southam et al., 1971) as a means to increase lifetime productivity (Dyrmundsson, 1973, 1981; Levine et al., 1978; Fogarty et al., 2007), but with the caveat that ewe lambs must be properly developed before breeding (Hume, 1939; Spencer et al., 1942). Lifetime lamb production is greater for ewes that lamb as yearlings than for ewes that lamb the first time as 2 yr olds (Bowstead, 1930; Spencer et al., 1942; Hulet et al., 1969; Baker et al., 1978; Levine et al., 1978; Fogarty et al., 2007). The BW of ewes lambing at 1 yr of age was lighter at yearling age (Cannon and Bath, 1969), end of 1st lactation (Griswold, 1932), and 18 mo (Spencer et al., 1942) compared with ewes that did not lamb at 1 yr of age, but BW were not different were not found at maturity. Lambing at 1 yr of age does not seem to adversely affect the ewes or their lambs (Fogarty et al., 2007). However, compared with ewes that lamb at 1 yr of age, ewes that lamb for the first time at 2 yr of age require an additional 12 mo of inputs without commensurate output, and overall production efficiency (i.e., ratio of useful output to total input) would be expected to be less. Indeed, in a recent survey of lamb producers (USDA, 2012), 61.1% of producers that acquired replacement ewe lambs indicated that early sexual maturity of ewe lambs was very or somewhat important. However, only 19.2% of producers indicated that National Sheep Improvement Program records, and thus, EBV were very or somewhat important. In lieu of genetic methods (e.g., EBV) to select for increased productivity at younger ages, producers apparently use phenotypic selection. Thus, a study was initiated at the USDA, ARS, US Sheep Experiment Station, to determine the additive genetic control of lambing percentage in yearling Targhee ewes.

Materials and Methods

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

Records of 3,103 ewe lambs born from 1989 to 2011 and retained as replacement lambs were analyzed in this study. Management of the ewe lambs after weaning and during breeding varied across years as experimental priorities shifted over time (Table 1). Before 2010, ewe lambs were weaned in late August, placed in feedlot pens for breeding, and managed in a feedlot until lambing. In 2010 and 2011, ewe lambs were weaned in early September with the same feedlot management through the winter until lambing. Before 2010, ewe lambs were penned with service sires during a 55-d breeding period. In 2010 and 2011, the breeding period was reduced to 34 d. During the earliest years recorded in the dataset, ewe lambs were bred in multisire pens, producing either purebred or crossbred lambs. Lambs resulting from these matings were not saved as replacements to the USSES purebred Targhee flock. Later, lambs were bred in single-sire pens, service sires of ewe lambs were recorded, and some replacement ewe lambs were saved to the Targhee flock. In 1998 and 2002,

mixtures of single-sire and multisire pens were used. From 2007 to 2011, ewe lambs were hand-mated to rams in an ongoing ewe puberty experiment, but due to experimental procedures to randomize service sires across ewe lambs, lambs could have been hand mated to more than 1 ram during the breeding season, precluding identification of a specific service sire. Because of the inability to correctly identify all service sires in years when service sires were not recorded or were in multisire pens, service sires of the ewe lambs were not included in this analysis. Instead, service sires, either known or unknown, were considered to be confounded within breeding pen. Consequently, any differences in lambing percentage due to service sire effects would be expected to be largely accounted for in breeding pen effects. Indeed, of the 39 known service sires, 84.7% were used as service sires for 1 yr only and were, thus, confounded within breeding pen.

The record of each yearling ewe contained sire, dam, actual weaning weight, breeding pen, age of dam, and lambing data. A lambing record for a ewe was a binomial trait (i.e., 1 or 0). Yearling ewes that delivered a live or stillborn, full-term lamb within approximately 10 d of their expected lambing date were coded as a success (1), and as a failure otherwise (0). We did not differentiate between ewe lambs that did not conceive and those that did conceive, but did not deliver a full-term lamb. Lambing percentage was defined as (n lambed/n mated) × 100.

The binomial structure of data from this study was not compatible with conventional statistical approaches for estimating heritability, calculating EBV, and determining the effectiveness of selection to improve some reproductive traits. Thus, statistical methods developed to transform binomial pregnancy data to a normal distribution (Evans et al., 1999; Doyle et al., 2000; Eler et al., 2002) were used. These methods generally increased heritability estimates for female reproductive traits and improved the characterization of genetic differences (i.e., EBV) among animals (Minick Bormann et al., 2006). Presently, EBV-based selection methods, with pregnancy EBV, are used to increase the probability that a heifer will calve at 2 yr of age (RAAA, 2011; AAA, 2012).

The ASREML software package (VSN International; Hemel Hempstead, UK) was used to estimate the heritability of yearling lambing percentage and calculate EBV. Fixed effects for yearling lambing percentage included year, breeding pen, and age of dam. Weaning weight of the ewe lamb was included as a covariate to account for the effect of growth rate on yearling lambing percentage.

There were 314 sires and 1,770 dams of the ewe lambs represented in the dataset (Tables 2 and 3). Of the 124 sires with less than 5 daughters, 15 sires had 1 daughter, 32 sires had 2 daughters, 37 sires had 3 daughters, and 40 sires had 4 daughters. Of the 3 sires with ≥ 50 daughters, individual sires had 51, 60, and 69 daughters represented in the dataset. The relationship matrix contained 6,877 unique animals.

Results and Discussion

Year affected lambing percentage ($P < 0.0001$), which varied from 26% in 2002 to 79% in 1996, and averaged 49.6% (Table 1). As expected, breeding pen (in effect, contemporary group) also affected ($P < 0.0001$) lambing percentage. The covariate, weaning weight, had a significant, positive linear affect on lambing percentage ($P < 0.0001$; Figure 1). After adjusting for ewe-lamb weaning weight, age of dam effects were not significant ($P = 0.15$).

The heritability estimate of yearling lambing percentage on the underlying scale was 0.18 ± 0.04. The EBV for lambing percentage of sires of ewe lambs on the observed scale ranged from -37 to 36% and averaged −1%. The 10th and 90th percentiles of sire EBV were −20 and 17%, respectively, which indicated that sires in the 90th percentile would be expected to produce daughters with an 18.5% greater likelihood of lambing at 1 yr of age than sires with EBV in the 10th percentile.

An experiment was initiated in the fall of 2012 to determine the efficacy of using EBV-based selection to increase yearling lambing percentage in Targhee sheep. Control and Selection lines were populated by randomly dividing ewes according to lambing percentage EBV within age groups among the lines. Rams with lambing percentage EBV > 1 SD from the mean were used to establish the Selection line, and rams with lambing percentage EBV within 1 SD of the mean were used for the Control line. In mid-February 2013, transabdominal ultrasound (Aloka 500 V instrument and 3.5 MHz convex transducer; Hitachi Aloka Medical, Ltd., Wallingford, CT) was used to determine the pregnancy status of 184 approximately 10.5-mo-old ewes. We considered pregnancy status to be an indicator of lambing percentage. Of the ewes evaluated, 52.1% were pregnant. Lambing percentage EBV ($P = 0.049$) explained 91% of the variation in pregnancy status. Yearling ewes with greater than average lambing percentage EBV had a pregnancy rate of 57.1 ± 6.5%, whereas ewes with less than average EBV had a pregnancy rate of 47.3 ± 6.5%. Even though, at this early-stage of the study, the numerical difference in pregnancy rate was not significant ($P = 0.24$), the significant relationship between lambing percentage EBV and pregnancy status provides the first indication that selection for yearling lambing percentage based on EBV may be an effective method for increasing yearling lambing percentage.

There is a dearth of published literature on using binomial analysis to estimate the heritability of yearling lambing percentage. However, heritabilities have been estimated for ewe age in days or months at first lambing as a continuous trait. In dairy sheep (El-Saied et al., 2005) and Dorset sheep (Lewis et al., 1998), the heritability of age at lambing was not significantly different from zero. In a study considering 33 different genetic groups of purebred and crossbred meat sheep, the heritability was 0.04 (Lobo et al., 2009). Gabina (1989) estimated heritabilities of 0.12 and 0.14 for 2 flocks. However, 2 studies reported larger heritabilities for the trait; Vanimisetti and Notter (2012) at 0.39 and Iniguez et al. (1986) at 0.31. The results from the present study compared favorably with the weighted average heritability (i.e., 0.10) of the previous studies.

However, except for the dairy sheep (El-Saied et al., 2005), the sheep in the other studies were in accelerated lambing systems. Unfortunately, the number of studies is not adequate to allow meaningful comparisons among heritability estimates for age at first lambing in dairy or meat sheep in intensive accelerated lambing systems with heritability estimates for lambing percentage in extensively managed meat sheep in rangeland systems, such as that in the present study. Nevertheless, our results support the premise that EBV-based selection can be used to improve lambing percentages of yearling Targhee ewes in an extensive, rangeland management system.

Implications

Results from this study indicate that sire effects on the ability of a ewe to lamb at approximately 1 yr of age are important and that genetic variation exists among ewe lambs in their ability to lamb at 1 yr of age.

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Table 1. Distribution of yearling lambing data by year

Year	Lambs, n[1]	Lambed, %[2]	Single-sire mated
1989	166	74.1	No
1990	170	61.1	No
1991	164	43.3	Yes
1992	197	31.4	No
1993	108	41.7	Yes
1994	90	37.8	Yes
1995	84	63.1	No
1996	123	79.0	No
1997	123	53.2	Yes
1998	144	70.8	Mix
1999	142	45.8	Yes
2000	135	40.7	Yes
2001	129	40.3	Yes
2002	119	26.0	Mix
2003	67	35.8	Yes
2004	118	68.6	Yes
2005	159	45.9	Yes
2006	163	56.4	Yes
2007	174	52.8	No
2008	177	33.8	No
2009	183	53.5	Yes
2010	135	34.1	No
2011	33	48.5	No

[1]Ewe lambs exposed to rams for breeding.
[2](n lambed/n mated) × 100.
[3]Mixtures of single-sire and multisire pens

Table 2. Number of daughter records by sire

Daughters	Sires, n
< 5	124
5 to 9	72
10 to 14	55
15 to 19	27
20 to 29	22
30 to 39	8
40 to 49	3
≥ 50	3

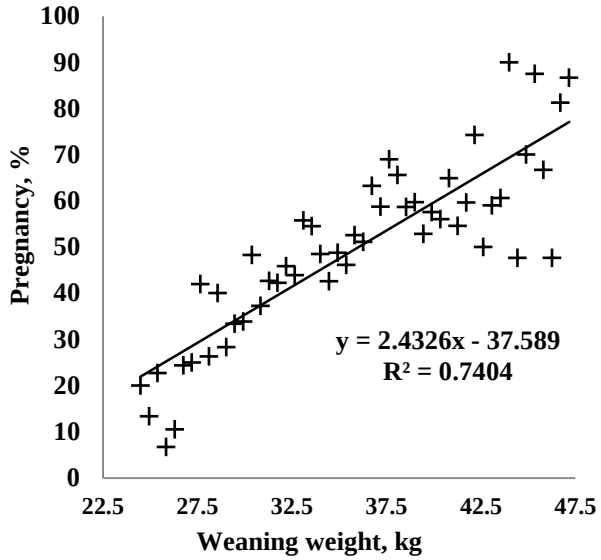
Table 3. Number of daughter records by dam

Daughters	Dams, n
1	939
2	430
3	222
4	102
5	48
6	25
7	3
8	1

Table 4. Distribution of daughter records by age of dam

Daughters, n	Records, %	Age of dam
175	5.6	1
728	23.5	2
725	23.4	3
556	17.9	4
412	13.3	5
294	9.5	6
186	6.0	7
27	0.9	8

Figure 1. Pregnancy percentage by weaning weight[1]



[1]Based upon weaning weight categories with 0.5-kg increments and ≥ 10 lambs present. Average weaning weight; 35.3 ± 5.4 kg.

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ESTIMATING ECONOMIC VALUES OF TRAITS FOR MATERNAL BEEF PRODUCTION SYSTEM

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ABSTRACT: Economic values are essential for constructing selection indexes for multiple traits selection where the goal is improve profit. The objective of this study was to estimate the economic values for a maternal beef production system at the Colorado State University Beef Improvement Center (BIC) and to determine the sensitivity of these values to changes in the production and marketing system. The estimation procedure consisted of four steps. First, the breeding, production and marketing system was specified. Next, costs and revenue were identified and economic information gathered. Third, economically relevant traits (ERT) influencing profit ability were determined and economic values were estimated and finally the breeding objective was developed on a per cow per year basis. Production, market, and economic information used in the study were obtained from the BIC and USDA. Twelve traits were included in the breeding objective profit equation which was subsequently used to estimate the economic value of each ERT with partial differentiation. Relative economic values for the ERTs were obtained by combining the economic value with the corresponding genetic variance. Sensitivity tests on economic values were performed by changing values of production traits (+/-10%) and economic information (+/-50%). The traits with the highest relative economic value were pre-weaning calf survival rate (SR), cow culling rate (CCR) and cow survival rate (CoSR). Results suggest that improvement on these traits would increase profitability. Sensitivity analysis indicated that changes in production variables have litte effect on estimation of economic values with correlations between original and changed scenarios all above 0.99. While changes in the feed price and hot carcass price do influence the economic values as evidenced by the negative correlations of -0.61 and -0.30 between economic values corresponding to original and changed prices (-50% feed price and +50% hot carcass price) respectively. The greatest differences in economic importance from alternative economic scenarios were seen for hot carcass weight and post weaning average daily gain (postADG).

Keywords: Breeding objective, Economic Value, Profit equation

Introduction

The general aim of agriculture producers is to maximize revenue while minimizing. For multiple traits selection, an index selection giving proper weight to each trait is more efficient than selection for one trait at a time or for several traits with an independent culling level for each trait. Since index selection can lead to greater increase in response (Hazel and Lush, 1942). Based on principles for construction of selection index as outlined by Hazel (1943), genetic parameters, phenotypic parameters and economic values are needed. Economic values reflect the economic importance of traits in a given production system. Ponzoni (1986) suggested economic values should be estimated based on information from the production and marketing system of a herd. Two methods were used to derive economic values including partial budgeting and partial differentiation of profit equation according to previous reports (Koots and Gibson, 1998; Fernandez-Perea and Alenda Jiménez, 2004). Also the efficiency of the selection index will be affected by changes in economic value (Smith, 1983).

The objective of this study was to estimate the economic values of traits for a maternal beef production system for the John E. Rouse Colorado State University Beef Improvement Center (CSU-BIC) of Saratoga, WY and to determine the sensitivity of these values to changes in the production and marketing system.

Materials and Methods

Production system. The breeding objective was developed on per cow per year basis using the procedures reported by Ponzoni and Newman (1989). Production and marketing information used in the study were based on the CSU-BIC. The production structure of CSU-BIC is shown in figure 1. Cow ages structure of the herd ranged from 1 to 16 years. Heifers which were not choosen as replacement heifers were assumed sold at age of 18 months of age; bulls (33.33% male calves) were sold at an age of 12 months; the slaughter age of animals (66.67% male calves) was set at 15 months. The replacement rate in the cow herd was 19.25% as calculated from 27,165 pedigree records from the CSU-BIC. Animals at the CSU-BIC are maintained through grazing in the summer and supplemental feeding of hay in the winter. The grazing period was from 1st May to 15th December every year.

Twelve economic traits related to cost and revenue of the herd was included in the breeding objective. They are cow survival rate (CoSR), cow calving rate (CR), calf survival rate before weaning (SR), birth weight (BW), weaning weight (WW), pre weaning average daily gain (preADG), post weaning average daily gain (postADG), calf survival rate post weaning (PSR), cow culling rate (CCR), cow weight (CoWT) and dressing percentage (DP).

Economic information. The average price for hay was assumed to be $260.5/ton and the cull cow price was set as $1.59/kg calculated from the USDA market reports (USDA-AMS, 2011; 2011-2012). The price of sale heifers ($1200/head), sale bulls ($1585/head) and hot carcass value ($3.14/kg) were based on the historical data of CSU-BIC.

Profit equation. A profit equation is a mathematic expression of the breeding objective. Generally the profit equation can be expressed as (Rewe, 2004):

$$P = \sum_{i=1}^{m} [n_i(R_i - C_i)X_i] - F$$

where *P* represent the total profit, m is the number of categories of animals in a herd, *n* is the number of animals for a trait in the *ith* class of animal *R* and *C* represent the revenue and cost per unit for trait *X* and *F* represents the fixed costs. Six animal categories (slaughter calves, sold heifers, sold yearling bulls, self-replacement heifers, and cows) were included in the profit equation. Returns were from the sale of heifers, bulls, cull cows and harvest animals; costs included feed cost, health cost, marketing cost and calving difficulty cost.

Derivation of economic values. Partial differentiation of the profit equation with respect to each trait with the value of other traits at their population means was used to estimate economic values. The relative economic values were obtained by multiplying the additive genetic effect standard deviation of each trait by economic values.

Sensitivity tests. Two sensitivity tests were performed: sensitivity of economic values to changing production levels and to economic condition. Economic values corresponding to +/- 10% of the original mean value of production traits (BW, WW, preADG, postADG and CoWT) and +/- 50% of the original economic information (feed price, hot carcass price, sale heifer price, sale bull price) were determined. Because the sale heifer and bull price used to estimate the base economic value were considered lower than recent price level, sensitivity tests were only done on +50% the original price. Correlations between economic values of original and varied production or economic information were calculated to study the effects.

Results and Discussions

Estimated economic values. Table 1 shows the economic values derived from the profit equation based on the maternal production system and the relative economic values. According to the results, the economic values for CoSR, SR, CR, PSR, WW, postADG, DP are all positive, which means a unit increase in these traits leads to increased return or decreased cost in the production system, while the economic values for CCR, BW, MY, CoWT and preADG are negative. Relative economic values can reflect the general economic importance of each trait. The most economically important traits in the study for the maternal beef cattle production system were reproductive related traits (SR, CCR and CoSR) as evidenced by the highest relative economic values. These results were similar to those reports from previous literature (Koots and Gibson, 1998; Fernandez-Perea and Alenda Jiménez, 2004; Kluyts et al., 2004; Rewe, 2004). Results provided evidence to suggest that improvement on these traits would highly increase profit.

Table 1. Marginal economic value and relative economic value of economically relevantly traits

Traits	Economic Value	Relative Economic Value
CoSR(%)	16.408	28.28
WW(kg)	0.550	4.71
BW(kg)	-0.221	-0.81
SR(%)	7.165	30.74
CR(%)	7.021	0.141
PSR(%)	4.698	8.10
CCR(%)	-6.327	-48.33
CoWT(kg)	-0.157	-8.53
MY(kg)	-0.072	-1.08
preADG(kg)	-13.703	-1.24
postADG(kg)	42.562	5.27
DP(%)	4.550	7.64

σ_A: square root of additive genetic variance

[a] BW:birth weight; WW:weaning weight; preADG:pre-weaning average daily gain; postADG:post-weaning average daily gain; SR:pre-weaning calf survival rate; MY:milk yield; CoWT: cow weight; CoSR:cow survival rate per year; CR:cow calving rate per year; PSR: post-weaning calf survival rate; USDAgrade: CCR:cow culling rate; DP:dressing percentage.

Sensitivity tests. Table 2 (above diagonal) shows the correlation between economic values corresponding to different production scenarios. According to the results, a +/- 10% change in production variable has limited effect on the economic value with the correlations above 0.99. The altered production characteristics would not lead to changing of economic rank of traits. This result indicated that the estimated economic values can be applied to different herds under the same economic environment.

Table 2 (below diagonal) shows the correlation between economic values corresponding to different economic scenarios. According to these correlations, the change of feed price and hot carcass price had large effects on economic values and resulted in negative correlations between original and changed price: -0.6076 and -0.2966. The economic value of postADG ranged from $160.35 to -$75.29 with changing feed price (+/-50% original price), and from -$26.31 to $111.37 with changing hot carcass price (+/-50% original price). This was because that postADG determined the feed cost and hot carcass returns in the profit equation. Thus, the varied economic conditions would highly influence the estimate of economic meaning that the estimated economic value cannot be applied to other herds with different economic environment.

Implications

Economic value can be appropriately estimated from the profit equation. The estimated economic value can be applied to herds with similar economic environment but not to varied economic circumstances. Further research on sequential effect of varying economic information on index weights should be done.

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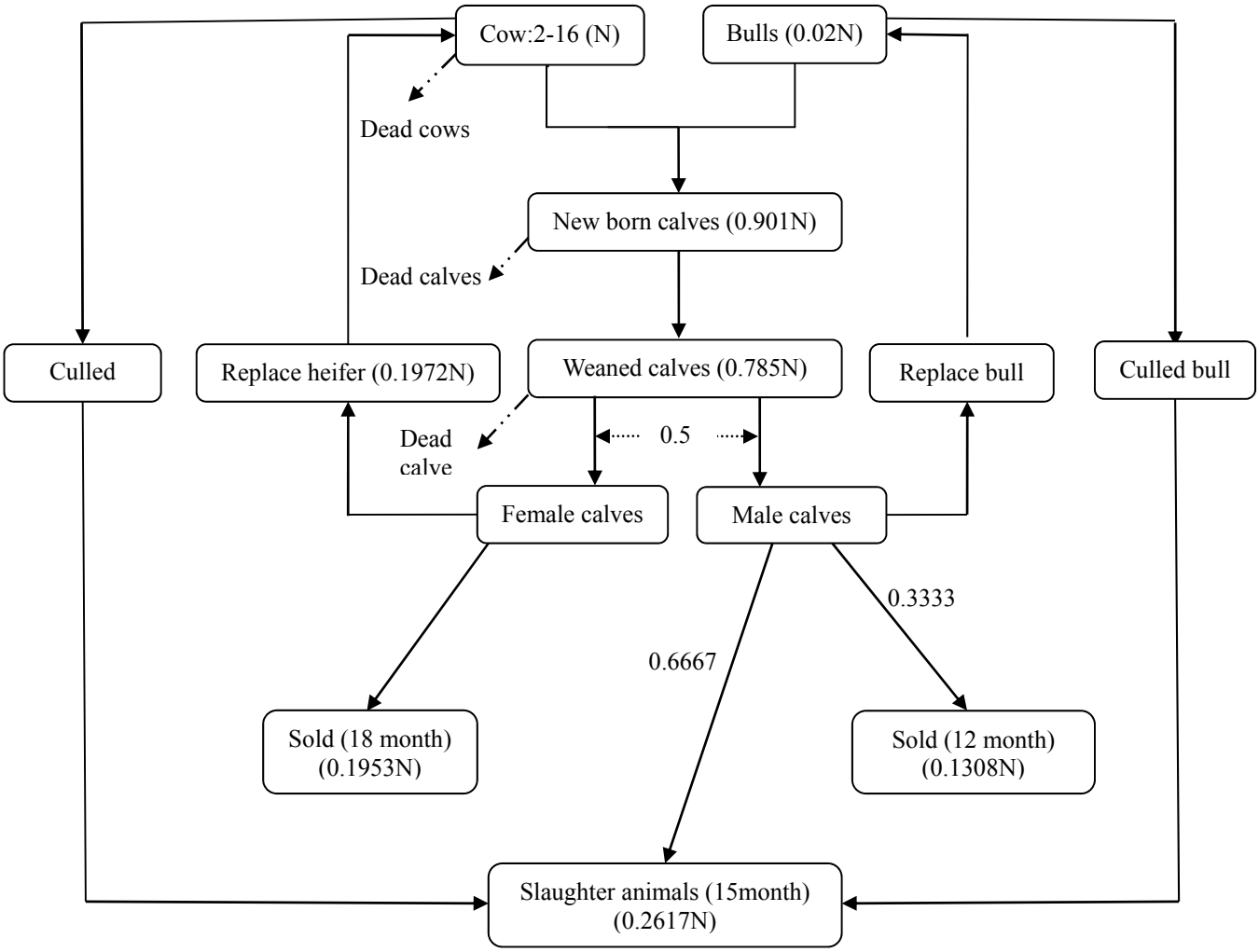

Figure 1. Production structure of John E. Rouse Beef Improvement Center of Colorado State University

Table 2. Correlation among economic values derived from different population mean of BW, WW, preADG, postADG and CoWT (above diagonal), and correlation among economic values derived from different feed, meat, heifer and bull price levels (below diagonal) for maternal systems based on information of John E. Rouse Ranch of Colorado State University Beef Improvement Center

EV[a]	Base	$BW_{0.9}$ /$pf_{0.5}$	$BW_{1.1}$ /$pf_{1.5}$	$WW_{0.9}$ /$phc_{0.5}$	$WW_{1.1}$ /$phc_{1.5}$	$preADG_{0.9}$ /$rhc_{1.5}$	$preADG_{1.1}$ /$rbc_{1.5}$	$postADG_{0.9}$	$postADG_{1.1}$	$CoWT_{0.9}$	$CoWT_{1.1}$
Base		1.0000	1.0000	0.9985	0.9979	1.0000	1.0000	0.9946	0.9960	0.9998	0.9998
$BW_{0.9}/pf_{0.5}$	0.9136		0.9998	0.9988	0.9975	1.0000	0.9998	0.9950	0.9955	0.9998	0.9998
$BW_{1.1}/pf_{1.5}$	-0.6029	-0.8752		0.9982	0.9982	0.9998	1.0000	0.9940	0.9964	0.9998	0.9998
$WW_{0.9}/phc_{0.5}$	-0.2902	-0.6525	0.9350		0.9929	0.9987	0.9983	0.9987	0.9898	0.9980	0.9988
$WW_{1.1}/phc_{0.5}$	0.9512	0.9939	-0.8185	-0.5712		0.9976	0.9981	0.9857	0.9997	0.9982	0.9973
$preADG_{0.9}/rhc_{1.5}$	0.9803	0.8407	-0.4834	-0.1455	0.8877		0.9999	0.9950	0.9956	0.9998	0.9998
$preADG_{1.1}/rbc_{1.5}$	0.9993	0.9072	-0.5911	-0.2764	0.9462	0.9817		0.9941	0.9963	0.9998	0.9998
$postADG_{0.9}$									0.9813	0.9936	0.9952
$postADG_{1.1}$										0.9965	0.9952
$CoWT_{0.9}$											0.9994
$CoWT_{1.1}$											

[a] Base: economic values from original production value and economic information; $BW_{0.9}$:economic values from10% less birth weight; $BW_{1.1}$: economic values from 10% more birth weight; $WW_{0.9}$:economic values from 10% less weaning weight; $WW_{1.1}$: economic values from 10% more weaning weight; $preADG_{0.9}$: economic values from 10% less pre-weaning average daily gain. $preADG_{1.1}$: economic values from 10% more pre-weaning average daily gain; $postADG_{0.9}$: economic values from 10% less post-weaning average daily gain; $postADG_{1.1}$: economic values from 10% more post-weaning average daily gain; $CoWT_{0.9}$: economic values from 10% less cow weight; $CoWT_{1.1}$: economic values from 10% more cow weight; $pf_{0.5}$: economic values from 50% less feed price; $pf_{1.5}$: economic values from 50% more feed price; $phc_{0.5}$: economic values from 50% less hot carcass price; $phc_{1.5}$: economic values from 50% more feed price; $rhc_{1.5}$: economic values from 50% more sale heifer price; $rbc_{1.5}$: economic values from 50% more sale bull price.

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RELATIONSHIPS AMONG TEMPERAMENT, IMMUNE FUNCTION, AND CARCASS MERIT IN BEEF CATTLE

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ABSTRACT: Cattle temperament has historically influenced selection decisions due to ease of handling. However, temperament may also influence economically relevant traits. The purpose of this study was to investigate relationships between temperament, Bovine Respiratory Disease (BRD) incidence, and resulting carcass merit in feedlot steers. Across a two year period, 2,870 crossbred steers were shipped from a single ranch source to a feedlot. At the time of feedlot placement, as well as at the time of reimplantation, temperament was measured via chute scores (CS) and exit velocity (EV). Blood samples were taken upon arrival to the feedlot to determine circulating concentrations of interleukin 8 (IL-8) and cortisol, both of which are involved in immune function. Performance traits, including weight and gains, were measured at feedlot placement (d 0), reimplantation (d 73-100), and again 59 to 70 days later. Recorded carcass data included hot carcass weight (HCW), USDA yield grade (YG), USDA quality grade, numeric marbling scores (MS), ribeye area, and lung scores. Statistical analysis was performed with SAS statistical software (SAS Inst., Inc., Cary, NC). Contemporary group (CG) included initial ranch unit, date of arrival to the feedlot, feedlot pen, and processing date. Fixed effects included in the model were pre-feedlot entry BRD treatment, post-feedlot entry BRD treatment, and CG. Cattle with higher CS at placement subsequently had higher BRD incidence rates ($P < 0.01$). There was a small, positive correlation between placement CS and blood cortisol concentrations ($r = 0.07$; $P < 0.01$), and cattle with higher cortisol concentration contracted BRD more often than their calmer peers ($P < 0.05$). Circulating IL-8 concentration had no influence on feedlot health. At the time of reimplantation, cattle that had been treated for BRD in the feedlot had lower average chute scores ($P < 0.001$). Placement CS had a small negative relationship with MS ($r = - 0.06$; $P < 0.01$). Exit velocity at reimplantation had a low, negative correlation with HCW and YG ($r = - 0.08$, $P < 0.01$ and $r = - 0.07$, $P < 0.01$). Overall, calmer cattle had better feedlot performance, less BRD incidence, and better carcass merit than their more excitable peers.

Key Words: beef cattle, temperament, BRD, disease

Introduction

Historically, cattle producers have selected for docile temperaments simply for management convenience because calmer animals are conducive to safe environments for their peers, as well as their handlers. As many producers would acknowledge, however, there seems to be a relationship between temperament and health, and calmer cattle tend to frequent the working chute for treatment of disease less often.

Positive correlations have been found in cattle between temperament traits (chute scores, pen scores, and chute exit velocities) and cortisol concentration in the blood, suggesting that more excitable cattle are easily stressed (Curley et al., 2006; Cooke et al., 2009). Additionally, Curley et al. (2007) found that easily excitable animals sustain elevated cortisol concentrations for a longer duration and had greater pituitary and adrenal responses following a stressor than calm cattle. Temperamental cattle have significantly higher mean temperament responses at all points (Oliphint, 2006). Higher basal serum cortisol concentrations may suggest that easily excitable cattle are chronically stressed (Curley et al., 2007), possibly resulting in a compromised immune response to disease pathogens, such as those necessary for Bovine Respiratory Disease (**BRD**) development.

Cattle diagnosed with BRD during the finishing phase have shown significantly lower average daily gains than untreated cattle (McNeill et al, 1996; Gardner et al., 1999; Bateman et al., 1990). Additionally, untreated steers yield higher marbling scores that result in a higher percentage of carcasses graded U.S. Choice and U.S. Select (Gardner et al., 1999).

This study was conducted to further investigate the relationships between cattle temperament (measured by chute score and exit velocity), immunological factors, and a range of economically relevant performance traits.

Materials and Methods

The Colorado State University Animal Care and Use Committee approved all experimental procedures (07-230A-01).

Crossbred steers were provided by a single source ranch with three locations in western Nebraska. In Year 1 (2007), 1,551 cattle were provided and in Year 2 (2008), 1,319 cattle were provided. In November of each year, cattle were shipped 536 km to a commercial feedlot in southeastern Colorado and were processed within two days of arrival to the feedlot. Initial processing included the administration of a radio frequency and visual identification tag, an oral and pour-on parasiticide, and a implantation of a growth promotant. At this time, a blood sample was taken and weight was recorded. Cattle were not vaccinated in Year 1 so that all animals could be equally challenged; however, 45 percent of animals experienced BRD. To avoid similar costs in Year 2, cattle were vaccinated for BRD with Pyramid 2 + Type II BVD and Presponse SQ (both from Boehringer Ingelheim, St. Joseph, MO).

Cattle were processed again at the time of reimplantation (~d 74) and a third time at approximately d 140. At both of these processing points, weights of the animals were recorded.

During the finishing phase, cattle were treated according to feedlot protocol if they exhibited more than two signs of BRD. Signs included lethargy, nasal and optical discharge, depression, cough, and a rectal temperature above 103.5º F. Cattle were secluded for 5-7 days post-treatment to be re-evaluated. At that time, those that were responding well to treatment were returned to their respective pens. Those that were still showing more than two signs of BRD were treated again.

Growth calculations included the gain between the first and second processing dates (**GAIN1**), the amount of gain between the second and third processing (**GAIN2**), and the total gain between the time of feedlot placement and the third processing.

Temperament was assessed using chute score (**CS**; Grandin, 1993; BIF, 2002) and exit velocity (**EV**; Burrow et al., 1988) at the first two processing dates. Once the steer was restrained in the chute, two evaluators assigned a CS to the animal. The CS scale ranges from 1 to 6, where calmer animals are on the lower end of the scale and the most aggressive cattle are at the upper end. The two appraised CS were averaged and CS was treated as a continuous variable for analysis. Once the processing was complete, the animal was released from the chute. At this time, the flight time, or the time it takes an animal to cover a defined distance (1.83 m), was recorded. Flight time was then converted to EV in units of m/s.

Cattle were harvested at d 225 on average at JBS Swift and Company plants in Dumas, TX and Greeley, CO in Year 1 and 2, respectively. At this time, carcass data was recorded. These data included a hot carcass weight (**HCW**), a calculated yield grade (**YG**), USDA quality grade, a marbling score (**MS**), ribeye area (**REA**), and lung scores. Two trained evaluators assigned a lung score of the aggregate lung. Lung scores were based on a scale of 0 to 3, where lower scores indicated less lung damage due to respiratory disease.

Assays were performed using the blood sample taken at the time of feedlot placement to determine cortisol and interleukin 8 (**IL-8**) concentrations in the blood. Both were measured using commercially available kits. Plasma cortisol was measured using a radioimmunoassay following the manufacturer's protocol (Coat-A-Count; Diagnostic Products, Los Angeles, CA). Interleukin 8 was measured using human ELISA kits that have been previously reported to cross-react with bovine IL-8 (Shuster et al., 1996, 1997; R&D Systems, Inc., Minneapolis, MN).

Statistical analysis of phenotypic measures was performed in SAS (SAS Inst., Inc., Cary, NC). Contemporary group (**CG**; n=11) accounted for differences in initial ranch unit, feedlot placement date, feedlot pen, and all processing dates. For all analyses, fixed effects were pre-feedlot BRD treatment and CG. To determine least squares means using the general linear mixed model, BRD treatment in the feedlot was also included as a fixed effect. The multivariate analysis of variance procedure was used to determine correlations among quantitative variables. Odds ratios were produced using the logistic regression procedure with qualitative response variables.

Heritabilities, genetic correlations, and repeatabilities were estimated with ASREML (Ver. 3.0, VSN International, Ltd., Hemel Hempstead, UK) on 2,871 animal records. The pedigree file included records of 7,177 animals with up to 7 generations. Data were analyzed using a multiple trait mixed animal model with animal as a random effect to estimate additive genetic merit. Fixed effects were the same as for the phenotypic analysis with the inclusion of permanent environment to determine repeatability estimates for temperament traits.

Results and Discussion

Table 1 shows phenotypic correlations between measures of temperament and all other measures collected. Cortisol had a weak but significant correlation with all temperament measures except EV at the time of feedlot placement (Table 1). Positive relationships between circulating cortisol concentrations and temperament have been reported previously, confirming that more excitable animals have significantly greater cortisol concentrations than their calmer peers (Cooke et al., 2007; Curley et al., 2006; King et al., 2006, Stahringer et al., 1990). Interleukin-8 was not significantly associated with any measure of temperament (Table 1).

Growth measures, including weights and gains, had few significant correlations with temperament traits, all of which were weak and negative (Table 1). This suggests that more excitable cattle will weigh and gain less throughout the finishing phase than their calmer peers. Carcass traits also had few significant correlations with measures of temperament, and all that were

significant were negative (Table 1). Appraised EV at the time of reimplantation was negatively associated with HCW and YG. Chute score at the time of feedlot placement had a small negative correlation with MS. Neither REA nor LUNG were associated with temperament regardless of the processing date.

Table 3 reports phenotypic correlations between measures of temperament at each of the first two processing points. All correlations were significant, and the strongest correlation was between EV at feedlot placement and EV at reimplantation (Table 3). Repeatabilities of the two temperament measures indicated that EV (r_p= 0.41 ± 0.02) was more repeatable than CS (r_p= 0.17 ± 0.02), which may be due to the objective nature of EV.

Heritabilities and genetic correlations of blood parameters and temperament traits are shown on Table 2. Cortisol is estimated to be lowly to moderately heritable, and IL-8 seems to be more influenced by genetics with a heritability estimate of 0.34 ± 0.07 (Table 2). The heritabilities of circulating coritisol and IL-8 concentrations have not been previously reported. Cortisol showed no significant genetic relationship with any of the temperament measures, nor with IL-8. Interleukin-8, however, had a negative correlation with EV at both time points, suggesting that cattle with genetics to be more temperamental will have genetics for decreased circulating IL-8 concentrations.

Temperament has been previously reported to be moderately heritable (Shrode and Hammack, 1971; Stricklin et al., 1980; Fordyce et al., 1988). Previous heritability estimates for CS specifically range from 0.15 to 0.30 (Burrow and Corbet, 2000; Kadel et al., 2006), while previous heritability estimates for EV range from 0.21 to 0.49(Burrow and Corbet, 2000; Burrow et al., 2001; Kadel et al., 2006; Nkrumah et al., 2007; Sant'Anna et al., 2012). The current study has estimates for the heritability of CS and EV at each of the first two processing times, and all estimates except EV at the time of feedlot placement fall within the respective previously estimated ranges (Table 2). Chute score appraised at the initial processing had a significant genetic correlation with EV at feedlot placement and CS at reimplantation, but not with EV at reimplantation (Table 2). The CS from the second processing was genetically correlated with EV at both time points as well (Table 2).

Table 4 shows estimated genetic correlations among temperament, immune, and carcass traits. Cortisol had a negative genetic correlation with both HCW and REA, suggesting that cattle exhibiting genetics potential for elevated cortisol levels will have decreased HCW and REA (Table 4). Interleukin-8 was positively genetically associated with HCW, MS, and YG (Table 4). Cortisol had a strong negative genetic relationship with BRD incidence in the feedlot segment, while IL-8 had a positive relationship with feedlot BRD incidence (Table 4). This indicates that cattle with genetics for greater cortisol concentrations upon feedlot placement may be inherently less susceptible to BRD, while those with genetics for greater IL-8 levels may be genetically predisposed to BRD. No previous literature has reported genetic relationships between carcass or immune traits and IL-8 or cortisol.

Chute score at the time of the first processing had a significant positive genetic correlation with HCW and BRD (Table 4). Exit velocity measured at the time of reimplantation also had a negative genetic relationship with HCW. Similar genetic relationships have been previously reported; Nkrumah et al. (2007) found moderate negative genetic associations between EV and HCW (r = -0.54). Exit velocity at the time of feedlot placement and CS at the second processing both had significantly positive correlations with REA (Table 4). Such results might indicate that innately more temperamental cattle will generally have genetics for larger REA. Temperament measures from the second processing point were negatively genetically correlated with MS; however, this was not true for temperament measures at the time of feedlot placement (Table 4). Exit velocity has previously been reported to have a genetic correlation with MS of 0.10 (Nkrumah et al., 2007). All measures of temperament were negatively genetically associated with YG (Table 4).

Exit velocity measured at the first processing date had a positive genetic correlation with the lung score, but other measures of temperament did not show similar significant relationships (Table 4). Temperament appraised at the second processing had negative genetic correlations with BRD incidence in the feedlot segment, suggesting that cattle with genetics to be more temperamental by the time of reimplantation will be less inherently susceptible to BRD than their peers.

Conclusions

Results from this study indicate that blood parameters (with the exception of IL-8) and temperament measures all have negative genetic relationships with BRD susceptibility in beef cattle, and more temperamental cattle do not seem to be inherently more susceptible to BRD incidence in the feedlot segment. Measures of temperament are genetically correlated with one another, and EV is estimated to be more repeatable that CS. Genetic correlations indicate that cattle with genetic potential to be more aggressive or fearful will have genetics for greater REA, reduced MS, and reduced YG.

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Tables

Table 1. Partial correlation coefficients of temperament traits with measures of immune function.

Trait	CS 1[1]	CS 2[2]	EV 1[3]	EV 2[4]
CORT[5]	0.0720**	0.0754**	0.0372	0.1120***
IL-8[6]	-0.0110	-0.0257	0.0157	0.0437
WT 1[7]	0.0255	0.0134	-0.0084	-0.0262
WT 2[8]	-0.0115	-0.0123	-0.0447	-0.1049***
WT 3[9]	-0.0233	-0.0404	-0.0588*	-0.1113***
GAIN1[10]	-0.0335	-0.0253	-0.0480	-0.1080***
GAIN2[11]	-0.0221	-0.0492*	-0.0336	-0.0344
TOTAL GAIN[12]	-0.0449	-0.0575*	-0.0656*	-0.1174***
HCW[13]	0.0185	-0.0236	-0.0371	-0.0799***
YG[14]	-0.0280	-0.0378	-0.0147	-0.0718**
MS[15]	-0.0643**	-0.0141	-0.0178	-0.0445
REA[16]	0.0226	0.0093	-0.0088	0.0125
LUNG[17]	0.0375	0.0040	-0.0090	-0.0111

[1]CS1= Average chute score at the time of feedlot placement (first processing)
[2]CS 2= Average chute score at the time of reimplantation (second processing)
[3]EV 1= Exit velocity at the time of feedlot placement
[4]EV 2= Exit velocity at the time of reimplantation
[5]CORT= Circulating serum cortisol concentration at the time of feedlot placement
[6]IL-8= Circulating interleukin 8 concentration at first processing
[7]WT 1= Body weight recorded at first processing
[8]WT 2= Body weight recorded at second processing
[9]WT 3= Body weight recorded at third processing
[10]GAIN1= Gain between the first and second processing
[11]GAIN2= Gain between second and third processing
[12]TOTAL GAIN= Total gain between the first and third processing
[13]HCW= Hot carcass weight
[14]YG= Yield grade
[15]MS= Marbling score
[16]REA= Ribeye area
[17]LUNG = Average lesion score of the aggregate lung
*P < 0.05; **P < 0.01; and ***P < 0.001.

Table 2. Heritabilities (on diagonal ± SE), phenotypic correlations (below diagonal ± SE) and genetic correlations (above diagonal ± SE) among temperament and immune traits in beef cattle

Trait	CORT	IL-8	CS1	CS2	EV1	EV2
CORT	0.23	-0.01	0.07	0.09	-0.11	0.11
	(0.06)	(0.16)	(0.17)	(0.19)	(0.19)	(0.16)
IL-8		0.34	-0.04	-0.08	-0.31	-0.22
		(0.07)	(0.15)	(0.17)	(0.17)	(0.15)
CS1			0.23	0.37	0.31	-0.02
			(0.05)	(0.17)	(0.17)	(0.17)
CS2				0.19	0.23	0.27
				(0.05)	(0.19)	(0.17)
EV1					0.17	0.73
					(0.05)	(0.11)
EV2						0.27
						(0.06)

Table 3. Correlation matrix with the partial correlation coefficients and associated significance of exit velocity and chute score at placement and reimplantation.

Trait	CS 1[1]	CS 2[2]	EV 1[3]	EV 2[4]
CS 1[1]	1.000			
CS 2[2]	0.2351***	1.000		
EV 1[3]	0.1406***	0.1803***	1.000	
EV 2[4]	0.1373***	0.2223***	0.4448***	1.000

[1]CS 1= Average chute score at the time of feedlot placement (first processing).
[2]CS 2= Average chute score at the time of reimplantation (second processing).
[3]EV 1= Exit velocity at the time of feedlot placement.
[4]EV 2= Exit velocity at the time of reimplantation.
*P < 0.05; **P < 0.01; and ***P < 0.001.

Table 4. Genetic correlations (± SE) among temperament, immune, and carcass traits in beef cattle

Trait	HCW	MS	REA	YG	LUNG	BRD
CORT	-0.34	-0.06	-0.19	0.08	0.16	-0.68
	(0.17)	(0.14)	(0.18)	(0.16)	(0.31)	(0.22)
IL-8	0.40	0.35	-0.01	0.37	-0.44	0.35
	(0.15)	(0.11)	(0.16)	(0.14)	(0.32)	(0.20)
CS1	0.18	-0.01	0.39	-0.21	-0.23	-0.01
	(0.17)	(0.13)	(0.18)	(0.16)	(0.33)	(0.22)
CS2	0.05	-0.16	0.28	-0.30	-0.28	-0.60
	(0.20)	(0.15)	(0.20)	(0.17)	(0.35)	(-0.22)
EV1	-0.12	-0.01	0.43	-0.46	0.36	-0.09
	(0.19)	(0.15)	(0.19)	(0.16)	(0.34)	(0.24)
EV2	-0.24	-0.14	0.17	-0.29	0.16	-0.34
	(0.17)	(0.13)	(0.17)	(0.14)	(0.29)	(0.21)

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IMPROVING GENOMIC PREDICTION IN SIMMENTAL BEEF CATTLE USING A MULTI-BREED REFERENCE POPULATION

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ABSTRACT: The objective of this study was to derive direct genomic breeding values (DGV) and evaluate their accuracies for 14 traits in Simmental beef cattle using single- or multi-breed reference populations derived from animals evaluated by the American Simmental Association. The traits were birth, yearling and carcass weights; calving ease direct and maternal; weaning weight direct and maternal; docility; fat thickness; marbling; rib eye area, shear force, stayability and yield grade. A total of 2,703 Simmental and 3,228 animals from non-Simmental breeds (mainly Black and Red Angus) were genotyped with the Illumina BovineSNP50 BeadChip. Genotyped Simmental animals were clustered into five groups using k-means clustering with the aim of increasing the within-group and decreasing the between-group pedigree relationships. Five-fold cross-validation was performed within-breed using four groups for training and the fifth group for validation. Multi-breed cross-validation used the same four Simmental groups for each of the five training runs, except that all the non-Simmental animals were included. In multi-breed analyses only the accuracies of Simmental predictions were of interest. Deregressed estimated breeding values were used as observations in a weighted analysis that estimated marker effects to derive DGV. Bivariate animal models were used for each trait to estimate the genetic correlation between trait and DGV as a measurement of the accuracy of genomic prediction. The accuracies of DGV training on Simmental alone ranged from 0.10 to 0.73 (average 0.47), and training on all breeds ranged from 0.18 to 0.91 (average 0.55). This is equivalent to an increase from 22% to 30% additive genetic variance explained. Genomic predictions were more accurate using multi-breed than the single-breed Simmental reference population for all traits except calving ease and weaning weight maternal. These results form the basis of current commercial implementation of DGV for American Simmental beef cattle.

Key words: accuracy, beef cattle, genomic breeding value, genomic selection, Simmental.

INRODUCTION

Assays can be used to genotype cattle for more than 50,000 single nucleotide polymorphisms (SNP) (Matukumalli et al., 2009). The resulting SNP markers can be used to produce direct genomic breeding values (DGV) for selection candidates that do not necessarily have phenotypes (Meuwissen et al., 2001). Selection using DGV could reduce generation intervals and increase genetic progress (Schaeffer, 2006). The accuracies of resultant DGV are key to successful application of this new technology in genetic improvement of any animal population as the genetic gain is directly proportional to the accuracy achieved. Size of reference population (a population of genotyped animals with phenotype used for estimating marker effects) is crucial factor influencing the accuracies of genomic predictions (Goddard and Hayes, 2009). It has shown that combining closely related populations could increase accuracies of genomic predictions (Lund et al., 2011). The objective of this study was to compare the accuracies of genomic predictions using single- or multi-breed reference population for American Simmental beef cattle.

MATERIALS AND METHODS

Genotype and Phenotype Data. A total of 2703 registered Simmental and 3228 non-Simmental animals (1766 Black Angus, 1268 Red Angus, 124 Gelbvieh, 37 Brangus, 31 Hereford and 2 Charolais) were genotyped with the BovineSNP50 BeadChip (Illumina, San Diego, CA) mainly at GeneSeek (Lincoln, NE). The details about the birth year distributions for the genotyped Simmental animals are in Saatchi et al. (2012). Deregressed estimated breeding values (DEBV), derived following Garrick et al. (2009) approach, were used as response variables to estimate SNP effects and their accuracies were used as weighting factors. This method results in DEBV that are free of parent average effects and the weights can be used to appropriately account for heterogeneous variance due to differences in reliabilities of individual and parent average EBV and therefore of corresponding DEBV. Expected progeny differences (EPD) and their Beef Improvement Federation (BIF) accuracies were obtained from American Simmental Association for all of the genotyped animals, their sires and dams were obtained from the American Simmental Association national cattle evaluation in October 2012. The EPD were transformed to EBV by multiplying by 2 and the corresponding reliabilities (R^2) were obtained as:

$$R^2 = 1 - (1 - BIF\_Accuracy)^2.$$

In total, 14 traits were analyzed (some traits were recorded in only some breeds, Table 1). The number of genotyped animals with DEBV varied among traits because some animals had no individual or offspring information contributing to their EPD. The number of genotyped animals with DEBV for each trait and each breed and the heritabilities of studied traits (reported by American Simmental Association) are in Table 1.

Statistical Model. Method "BayesC" (Kizilkaya et al., 2010) was used to estimate marker effects for genomic prediction. This method assumes that non-zero SNP effects are drawn from a distribution with constant variance but that some known fraction of markers (π) have zero effect. Habier et al. (2011) showed that BayesC is less sensitive to prior assumptions than BayesB. For each trait, the following model was fit to the DEBV data for training:

$$y_i = \mu + \sum_{j=1}^{k} z_{ij} u_j + e_i \ ,$$

where y_i is the DEBV for animal i, $\mu$is the population mean, k is the number of marker loci in the panel, z_{ij} is allelic state (i.e., number of B alleles from the Illumina A/B calling system) at marker j in individual i, u_j is the random effect for marker j, with u_j □ $N(0, \sigma_u^2)$ (with probability 1 - π) or $u_j = 0$ (with probability π), and e_i is a residual with heterogeneous variance, depending on the reliability of the DEBV information for animal i (Garrick et al., 2009). Parameter π was assumed to be 0.95 for all analyses. Markov chain Monte Carlo (MCMC) methods with 41 000 iterations were used to provide posterior mean estimates of marker effects and variances, after discarding the first 1000 samples for burn-in. The previous estimates of genetic and residual variances (Saatchi et al., 2012) were used for constructing priors for genetic and residual scale parameters.

The DGV for individual i within a validation set was derived as the sum over all k markers of posterior means of predicted SNP effects, as estimated in the training set, multiplied by the number of copies of the B allele:

$$DGV_i = \sum_{j=1}^{k} z_{ij} \hat{u}_j$$

where DGV_i is the DGV for individual i in the validation dataset, z_{ij} is the marker genotype of individual i for marker j, and $\hat{u}_j$ is the posterior mean effect of marker j over the 40,000 post burn-in samples. All analyses were performed using GenSel software (Fernando and Garrick, 2010).

Accuracies of Genomic predictions. The accuracy of DGV for each trait was evaluated by pooling estimates from a 5-fold cross-validation strategy. The genotyped Simmental animals were first divided into five unequally-sized mutually exclusive groups using K-means clustering method. Details about the clustering of genotyped Simmental animals are in Saatchi et al. (2012). Single-breed training analyses were performed by excluding one group when estimating marker effects, which were then used to predict DGV of individuals from the omitted group (validation set). This resulted in every animal having its predicted DGV obtained without considering its own DEBV. In multi-breed cross-validation, the same four Simmental groups for each of the five training runs were used, except that all the non-Simmental animals were included in training analyses. In multi-breed analyses only the accuracies of Simmental predictions were of interest.

We applied a weighted bivariate animal model using the DGV of genotyped Simmental animal, and their DEBV to estimate variance and covariance components for each of the studied traits. The purpose of fitting this model was to estimate the genetic correlation between the trait (T) and its respective DGV ($r_{g(T,DGV)}$). This trait-DGV genetic correlation is required to pool DGV and traditional EBV in national genetic evaluation (MacNeil et al., 2010). The further details are in Saatchi et al. (2012).

RESULTS AND DISCUSSION

The estimated heritabilities of DGV and the estimated trait-DGV genetic correlations are shown in Table 2. Heritabilities of DGV were 1.00 for most traits in both scenarios. Heritabilities of 1 are expected for perfectly inherited attributes, such as SNP genotypes or linear functions of SNP genotypes as shown by many studies (MacNeil et al., 2010; Saatchi et al., 2011; Saatchi et al., 2012; Saatchi et al., 2013).

The estimates of genetic correlation from training on Simmental alone ranged from 0.10 to 0.73 (average 0.47), and from training on all breeds ranged from 0.18 to 0.91 (average 0.55). Genomic predictions were more accurate using multi-breed than the single-breed Simmental reference population for all traits except calving ease and weaning weight maternal. This demonstrates the benefit of using multi-breed reference population for American Simmental beef cattle. Lund et al. (2011) showed that the combining reference populations from four European Holstein populations increase the average of accuracies of DGV by 0.32% (or increase of reliability of 10%), compared with those obtained from national reference populations alone (same breed but different countries). Less improvement in accuracies has been reported using multi-breed reference population of less related populations of Holstein and Jersey breeds (Hayes et al., 2009). In our study, an increase from 22% to 30% additive genetic variance explained, obtained by using multi-breed reference population for American Simmental beef cattle. This may reflect this reality that registered Simmental animals have a heterogeneous genetic background admixed with other beef cattle breeds, as

American Simmental Associations allow registration of crossbreds with other beef cattle breeds.

IMPLICATIONS

This study applied genomic prediction to US Simmental beef cattle using single-breed and multi-breed reference populations. The accuracies of genomic predictions were established based on the genetic correlation between the trait and its DGV. Genomic predictions were more accurate using multi-breed than the single-breed Simmental reference population for all traits except calving ease and weaning weight maternal (increase from 22% to 30% additive genetic variance explained). This demonstrates the benefit of using multi-breed reference population for American Simmental beef cattle.

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Table 1 Heritability (h^2), number of genotyped animals with DEBV for each trait in each of breed[1]

		Simmental	Black Angus	Red Angus	Gelbvieh	Other breeds
Trait	**h^2**	N=2703	N=1766	N=1268	N=124	N=70
Birth weight	0.42	2664	1691	1224	117	47
Calving ease direct	0.12	2443	1692	1207	99	36
Calving ease maternal	0.13	2441	1686	1204	99	34
Carcass weight	0.40	2663	1686	1221	117	47
Docility	0.35	528	238	61	1	-
Fat thickness	0.35	2463	1572	1117	16	20
Marbling	0.54	2439	1588	1120	60	20
Rib eye muscle area	0.46	2435	1596	1110	60	20
Shear force	0.40	1045	56	-	3	-
Stayability	0.21	563	-	-	-	-
Weaning weight direct	0.30	2663	1691	1224	117	47
Weaning weight maternal	0.14	2661	1683	1187	117	47
Yield grade	0.40	2574	1641	1129	82	39
Yearling weight	0.29	2663	1691	1225	117	47

[1]Heritabilities reported by American Simmental Association

Table 2 Estimates of heritability of DGV and of genetic correlations between trait and its DGV in Simmental animals using single- and multi-breed reference populations[1]

	Single-breed reference		Multi-breed reference	
Trait	h_{DGV}^2	$r_{g(T,DGV)}$	h_{DGV}^2	$r_{g(T,DGV)}$
Birth weight	1.00±0.00	0.67±0.03	0.99±0.02	0.73±0.03
Calving ease direct	1.00±0.00	0.46±0.02	1.00±0.00	0.49±0.02
Calving ease maternal	0.99±0.02	0.31±0.02	1.00±0.03	0.29±0.02
Carcass weight	1.00±0.00	0.61±0.04	0.97±0.02	0.75±0.03
Docility	1.00±0.00	0.10±0.09	1.00±0.00	0.18±0.11
Fat thickness	0.99±0.02	0.19±0.02	1.00±0.00	0.26±0.02
Marbling	1.00±0.00	0.60±0.04	1.00±0.00	0.69±0.04
Rib eye muscle area	1.00±0.00	0.55±0.05	0.97±0.03	0.72±0.05
Shear force	1.00±0.00	0.52±0.08	1.00±0.00	0.60±0.08
Stayability	1.00±0.00	0.51±0.05	1.00±0.00	0.51±0.05
Weaning weight direct	1.00±0.00	0.56±0.04	0.95±0.02	0.63±0.03
Weaning weight maternal	1.00±0.00	0.32±0.03	1.00±0.00	0.28±0.03
Yield grade	1.00±0.00	0.73±0.09	1.00±0.00	0.91±0.11
Yearling weight	1.00±0.00	0.45±0.02	0.95±0.03	0.67±0.03

[1]Heritability of DGV (h_{DGV}^2±SE) and genetic correlations between trait and its DGV ($r_{g(T,DGV)}$±SE) estimated from bivariate animal models in Simmental animals

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FACTORS INFLUENCING SELECTION FOR PULMONARY ARTERIAL PRESSURE IN CATTLE AT HIGH ELEVATIONS

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ABSTRACT: High altitude disease (HAD) in cattle, commonly named "Brisket Disease", typically occurs at elevations over 1,524 m. The incidence of HAD in cattle native to high elevation ranges from 0.5 to 5%. This increases to a range of 10 to 50% when cattle are moved from low to high elevations (i.e. non-native cattle). High altitude disease can be lethal when animals are unable to cope with these changes in elevation. Given increased mortality rates of cattle at high elevation, identifying tolerant breeding stock is economically relevant. Pulmonary arterial pressure (PAP) is seen as an indicator of an animal's susceptibility to HAD and can be used as a selection tool to identify animals that are less susceptible to HAD given PAP has been reported to be highly heritable. The objective of this study was to identify environmental factors influencing PAP measures and to determine whether there are breed differences for PAP. Data consisting of birth year, pen, breed, yearling age, yearling weight and PAP were obtained from the 4-Corners Bull Test located at the San Juan Basin Research Center (elevation 2,466 m). A subset of 2,107 bull records from 1983 to 2005 with PAP measures were used. Fourteen breeds were represented over fourteen years of relevant data. The R statistical package was used for linear prediction analyses. We investigated the effects of birth year, pen, breed, yearling age, and yearling weight on yearling PAP. Yearling weight ($P > 0.05$) was not found to account for significant variation in PAP and was therefore removed from the model. There was a 13.6 mmHg range between breeds with the lowest adjusted PAP estimate to those with the highest adjusted PAP. Angus x Gelbvieh crosses had the lowest adjusted PAP and Simmental bulls had the highest. Results show that with each unit increase of yearling age, PAP increased by 0.03 mmHg ($P < 0.01$). The results of the study indicate that breed was an important factor influencing PAP measures in bulls developed at high altitude ($P < 0.001$). Using breed in selection criteria for high elevation beef production systems could be helpful in reducing the incidence of HAD.
Keywords: breed, cattle, high altitude disease, pulmonary arterial pressure

Introduction

High altitude disease (HAD), commonly known as "Brisket Disease", has been a concern for cattle producers within the Rocky Mountain region for approximately a century. The disease is characterized by the accumulation of edematous fluid in the tissues covering the parasternal muscles, also known as the brisket region (Holt et al. 2007). This fluid accumulation is due to increased vascular hydrostatic pressure and subsequent loss of fluid into the extravascular spaces, such as the pericardium of the body (Holt et al. 2007). High altitude disease has been found to occur in cattle residing at elevations of 1,524 m or higher and the condition results in poor performance and often death. An indicator of this disease is pulmonary arterial pressure (PAP) (Holt et al. 2007).

Pulmonary arterial pressure is heritable (Enns et al., 1992) and therefore can be used to make breeding decisions. The PAP measurements reflect the animal's ability to adapt to high-elevated regions. According to Holt et al. (2007), cattle over 12 months of age with PAP values less than 41 mmHg are suitable for breeding stock in high elevations and have a reduced risk for developing HAD. The heightened concern from this disease stems from profit losses associated with increased death loss in cattle unable to cope with changes in altitude. The incidence of HAD in cattle depends greatly on genetic selection pressure for elevated PAP measurements (Enns et al., 1992). For animals native to high elevation, the incidence of HAD ranges from 0.5% to 5%. For non-native cattle, the incidence is reported to increase to 10% to 50% (Will et al., 1975; Rhodes 2005; Holt et al. 2007; Neary 2013).

Being able to identify animals less susceptible to HAD is of economic significance to beef production systems in the Rocky Mountain region. The objective of this study was to evaluate environmental factors and breed effects in PAP measures in historic bull test data from Southwest Colorado.

Materials and Methods

Historical data was obtained from the 4-Corners Bull Test located at the San Juan Basin Research Center (SJBRC), near Hesperus, Colorado from 1948 to 2005. Bull tests were conducted and data was collected based on SJBRC guidelines. Bulls were annually consigned to SJBRC from the Rocky Mountain region. The SJBRC resides at an elevation of 2,466 m. Typically adaptation and gain tests lasted between 84 and 140 days, depending on the year of test. Pulmonary arterial pressure measurements were taken at the end of the testing period, when the bulls were yearlings.

The data for this study consisted of birth year, pen, breed, yearling age, yearling weight, and PAP. Pulmonary arterial pressure measurements were recorded for 14 years of the test; specifically, years 1983, 1989, and 1993 to 2005, excluding 2003. The original data included bulls of 21 breeds; however, breeds of cattle with less than 5 bulls with PAP measurements were omitted from the analyses. The final data set contained 2,107 bull records with fourteen breeds of cattle including: Charolais, Angus x

Gelbvieh, Angus, Composite, Gelbvieh, Hereford, Limousin, Maine-Anjou, Polled Hereford, Red Angus, Salers, Simmental, System1 Composite and other (breed unknown).

The CAR package in R was used for linear prediction analyses (GNU General Public Library, R. Development Core Team). The model was fitted based on the factors available within the dataset and importance in relation to PAP measurements. Specifically birth year, pen, breed, yearling age and yearling weight effects on yearling PAP were tested as sources of variation. Yearling age was used as a covariate in the model. In the results, yearling weight was not significant ($P > 0.05$) and was therefore removed from the model.

Results and Discussion

The model of birth year, pen, breed, and yearling age effects on yearling PAP revealed these terms were significant ($P < 0.01$) predictors of PAP (Table 1). For yearling age, each day increase of yearling age increased PAP by 0.03 mmHg ($P < 0.01$). The birth year solutions had a range of 7.7 mmHg and the effect of pen had a range of 12.6 mmHg.

There was a 13.6 mmHg range between breeds with the lowest adjusted PAP estimate to those with the highest adjusted PAP (Table 2). Angus x Gelbvieh crosses were found to have the lowest adjusted PAP values of all breeds, rendering them less susceptible to HAD. Simmental bulls were found to have the highest adjusted PAP values of all breeds compared, rendering them more susceptible to HAD. Breed was found to be a highly significant factor ($P < 0.001$) in the model influencing PAP measurements for bulls developed at high altitude at the SJBRC.

To our knowledge there has been no previously reported information regarding breed difference in PAP.

Implications

The results of the study suggest that breed selection could be advantageous in reducing the susceptibility of cattle to HAD through reduced pulmonary arterial pressure measurements in mountainous beef production systems. This could directly affect the economic impact HAD has on beef production systems in the Rocky Mountain region. With increasing interest in PAP measurements as they relate with HAD and other respiratory and cardiovascular diseases, further research can be conducted to determine more extensively the factors of economic and relevant impact to beef production systems.

Table 1. Analysis of variance for the final model.
Response: Pulmonary arterial pressure

	Sum Sq.	Df	F Value	Pr (>F)	
Intercept	27774	1	193.8519	2.20E-16	***
Birth Year	14033	13	7.534	1.02E-14	***
Pen	10986	44	1.7426	0.001923	**
Breed	14147	13	7.5955	7.28E-15	***
Age [A]	1000	1	6.9817	8.30E-03	**
Residuals	291563	2035			

Significance codes: ***$P < 0.001$; **$P < 0.01$ *$P < 0.05$
[A] Used as a covariate

Table 2. Results of model means and coefficients for pulmonary arterial pressure by breed.

Breed	Unadjusted Mean	Estimate	Standard Error
Intercept [A]	45.138	50.140	3.601
AN/Gelb	44.000	-8.196	4.253
Angus	47.656	-0.297	1.351
Composite	43.051	-4.447	1.240
Gelbvieh	46.038	1.271	1.599
Hereford	42.685	-4.830	1.404
Limousin	43.769	-1.402	2.078
Maine Anjou	47.200	1.546	5.670
Other [B]	43.506	-2.754	1.749
P. Hereford	42.316	-3.378	1.708
Red Angus	46.723	2.889	1.403
Salers	39.500	-7.587	3.985
Simmental	53.120	5.440	1.819
System1	46.921	1.817	1.300

[A] Zeroed for Charolais breed
[B] Breeds unknown

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ESTIMATES OF GENETIC PARAMETERS OF CARCASS TRAITS IN THE PROGENY FROM CROSSES INVOLVING CHAROLAIS, BRAHMAN, BRANGUS, HEREFORD, HOLSTEIN, AND ANGUS INHERITANCE

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ABSTRACT. The objective of this study was to estimate descriptive statistics, genetic parameters of heritability and genetic correlations among carcass traits in the progeny (n=511) from crosses involving Charolais, Brahman, Brangus, Hereford, Holstein, and Angus (inheritance) are presented. Data came from a commercial beef herd. Cattle were slaughtered at a mean age of 440d. Each breed or cross was stratified by weight and age to contribute to each group. The feeding period for the three groups was 211,195, and 180 days, respectively. All cattle were slaughtered at a commercial slaughter facility at a mean age of 440d. The traits evaluated included final weight at slaughter, hot carcass weight, adjusted fat thickness, kidney pelvic and heart fat, rib eye area, yield grade, and marbling score. Separate analysis for each trait used SAS (1989). The analytical model included: Fixed main effects of sex, slaughter group, sire breed, dam breed, sire x dam, sex x sire, and sex x dams interactions**;** random nested components of sires within sire breed and dams within dam breed and the residual. Charolais crosses were the heaviest and consistent, average of all crosses =1118.33 lb versus 998.37 lb of all crosses. Sex effects were highly significant (P<0.001) for final weight at slaughter, and yield grade but not for marbling. Slaughter group was highly significant (P<0.01) for final weight at slaughter. Sire breed effects were (P<0.05) for final weight at slaughter and highly significant (P<0.001) for yield grade. Sires nested as random components within sire breed were non significant for final weight at slaughter, yield grade, or marbling score. Dam breed effects resulted (P<0.001) for all these carcass traits. Dam breed effects nested within dam breed were not significant for final weight at slaughter, yield grade, or marbling. Estimates of heritabilities (h2 =.45, h2 = .47, and, h2 =.36) corresponded to final weight at slaughter, yield grade, and marbling score, respectively. Estimates of genetic correlations among these traits were (rg=.43, rg =.45, and rg=-.21).

Keywords: genetic parameters, carcass traits, crossbreeding, beef cattle.

Introduction

The objective of the beef cattle industry is to synchronize production and carcass characteristics of breed resources with the production resources that are most economical to provide to maximize economic efficiency. Breed differences in bioeconomic traits are an important genetic resource and can be used to achieve and maintain performance levels that are optimum for different production and marketing situations. In addition to using breed differences to optimize production and carcass traits or to meet specific targets, the mating system should be organized to achieve and maintain high levels of heterosis or hybrid vigor (Gregory et al., 1995). The objective of this study was to estimate: descriptive statistics, genetic parameters of heritability and genetic correlations among carcass traits in the progeny from crosses involving Charolais, Brahman, Brangus, Hereford, Holstein, and Angus, inheritance.

Materials and Methods

Carcasses traits of (n=511) crossbred steers and heifers in different proportion inheritance were studied. Data came from a commercial beef herd in Baja California México. Cattle were slaughtered at a mean age of 440 d. Each breed or cross was stratified by weight and age to contribute to each group. The feeding period for the three groups was 211, 195, and 180 d, respectively. All cattle were slaughtered at a commercial slaughter facility. The traits evaluated included final weight at slaughter (FWS), hot carcass weight (HCWT), adjusted fat thickness (AFT), kidney pelvic and heart fat (KPH), rib eye area (REA), yield grade (YG), and marbling score (MS). The estimate of genetic parameters of heritability and genetic correlations among carcass traits in the progeny from crosses involved Charolais, Brahman, Brangus, Hereford, Holstein, and Angus. The objective was to estimate: descriptive statistics, genetic parameters heritability and genetic correlations between carcass traits as (FWS), (YG), and (MS). Carcasses were evaluated by USDA quality and yield criteria.

Statistical analyses

Separate analyses for each trait was using SAS (1989). The analytical model included: fixed main effects of sex, slaughter group, sire breed, dam breed, sirexdam, sex x sire, and sex x dams interactions; random nested components of sires within sire breed and dams within dam breed and the residual. All posible interactions among main effects were tested, and those found not significant were deleted from final models.

Results and Discussion

Descriptive statistics of carcass traits (FWS), (HCWT), (AFT), (KPH%), (REA), and (YG), and (MS), are presented in Table 1. As shown Charolais crosses were the heaviest at slaughter average of all crosses = 1118.33 lb versus 998.37 lb of all crosses out Charolais. Table 1 also shows that the averages for carcass traits (0. 61 in, 2.29%, 11.48 in2, 2.77, and 342) were to (AFT), (KPH%), (REA), (YG), values and (MS), respectively. Table 1 also shows that lowest values in (FT) corresponded to crosses involving HxA, HxB, HxBr, and ChxB inheritance, intermediate values in (FT) were to crosses ChxBr, BrxBr, BxBr, and ChxA, while highest values in (AFT) involved HxH, BxB, and BrxA inheritance. Table 1. shown that lowest values in (KPH %) involved BxBr, HxB, and BxB inheritance, intermediate values were from crosses ChxB, ChxBr, HxBr, and BrxBr, highest values in (KPH%) corresponded to Holsein x Holstein, and crosses BrxA, HxA, and ChxA inheritance.

Highest values in (REA) corresponded to ChxB, ChxA, HxBr, and ChxBr inheritance, intermediate values in (REA) were for HxA, BrxBr, and HxB, crosses. Lowest values in (REA) corresponded to crosses involving BxB, BxBr, HxH, and BrxA inheritance.

Highest (YG) values corresponded to crosses involving BrxA, BrxBr, ChxA, and HxA inheritance. Intermediate (YG) values corresponded to crosses, HxB, ChxBr, HxB,and HxBr, respectively. Lowest values in (YG) were for carcasses of progeny involving inheritance Holstein x Holstein, BxBr, and Brahman x Brahman. Even though lean retail yield is a moderately heritable trait, it would require a significant amount of time to change it by within breed selection. It has been estimated that if breeders were to hold other traits constant and select for improved retail product yield alone, it would take nearly 15 years to change population by one yield grade (Cundiff, 1987).

Table 1. also shows that highest values in marbling involved inheritance of HxA, HxBr, BrxBr, and ChxA, respectively. Intermediate values in (MS) were for BxBr, BrxA, ChxB, and ChxB. Lowest values in (MS) were to crosses involving a major proportion of Bos índicus proportion ChxBr, Holstein x Holstein, and BxB inheritance. These findings in agreement to (Koch et al., 1982b and Crouse et al., 1989).

Cundiff, (2000) when compared Continental (C) and British (B) breeds in different proportion for carcass quality traits of F1 steers harvested at 444 days of age. The percentages of Choice and Yield Grade 1 and 2 were (30 and 89; 43 and 83; 56 and 56; 66 and 52; and 70 and 38) for inheritance of Continental 100%, .75Cx.25B, .50Cx.5B, .25 C x.75 B , and 100% British, respectively.

Shackleford et al. (1992) suggest that an alternative to selection for marbling, is selection for low calpastitan activity. Calpastitan inhibits Calpain which plays an important role in the effect of aging on beef tenderness. The authors involved (n=555) steers of diverse breed and crosses, Calpastatin was highly heritable (h2 =.70) and a high genetic correlation (rg=.58). Selection for low calpastatin activity may be particularly useful in Bos indicus breeds and composites which have shown especially high levels of shear force (SF) and high levels of calpastitan activity (Wipple et al ., 1990; Shackelford et al., 1991).

Mean squares for carcass traits are presented in Table 2. Sex effects, were highly significant (P<0.001) for (FWS), (YG), and (MS). Table 2. Also shows that slaughter group was highly significant for (FWS); however non significant differences (P>0.05) were found for (YG) and (MS). Sire breed effects were significant (P<0.05) for (FWS) and highly significant (P<0.001) for (YG); however sire breed effects were not significant (P>0.05) for marbling.

Effects of sires nested within sired breed are also shown in Table 2. Non significant differences (P>0.05) were found for final (FWS), (YG) or marbling scores. Table 2. Also shows highly significant (P<0.001) differences for (FWS) and (YG); however not significant effects were found for (MS) attributable to dams breed effects.

Table 2. Also shows that dam breed effects resulted highly significant (P<0.001) for both (FWS) and (YG); however not significant (P>0.05) differences were found for marbling. It suggests that contribution of dams should be considered in breeding plans of breeding plans. Half of the advantages of crossbreeding depend on their use (Cundiff, 1977).

Dams nested within cow breed effects are also presented in Table 2. Non significant effects (P>0.05) were found for (FWS), (YG), and (MS). Table 2. Also shows highly significant effects (P<0.001) due to sire x dam interaction for (FWS). The non significant effects of other interactions were deleted.

Genetic parameters

Table 3. Shown estimates of heritabilities and genetic correlations of carcass traits in this study were (h2 =.45, h2 = .47, and, h2 =.36) corresponded to (FWS), (YG), and (MS), respectively. Estimates of correlations among these traits were (rg=.43, rg =.45, and rg=-.21), respectively.

Marston et al. (1999) reported average estimates of heritability (h2 =.43) for marbling. These results in most cases in agreement with literature estimates.

Koots et al. (1994) based of (n=287) global studies covering 45 years (1946 to 1991) and Marshall (1994) of 13 US sources covering 15 years (1978 t0 1993) and (n=43,177) progeny in independent studies both summarized published heritability estimates for carcass traits age–or time-on-feed constant basis (h2 = .41, h2 = .23, h2 =.39, h2 =.94, h2 = na , h2 =.42, h2 =.47, h2 =.55, h2 =.38, h2 =.29; and h2 =na, h2 =.41, h2 = na, h2 =.44, h2 =.57, h2 =.37, h2 =.36, h2 = na, h2 = .35, and h2 =.37), market weight, carcass weight, percentage of cuts, (FT), percentage of fat cuts, (REA) , retail yield cuts, lean percentage, (MS), and shear force, respectively (Koots et al. 1994; and Marshall 1994).

ISU and American Angus Association reported heritabilities and genetic correlations of carcass and ultrasound traits involving inheritance of Angus were estimated (h2 =.37, h2 = .28, h2 = .24, and h2 =.24; and h2 =.37, h2 =.36, h2=.37, and h2 =.36) for carcass and ultrasound, for (MS), (REA), (FAT), and percentage retail product, respectively. Estimates of correlations among these traits were (rg=0.77 , rg =0.75, and rg =0.71), respectively.

Lawrence et al. (2001) analyzed (n=60,625) data from cattle for relationships among carcass traits. The authors found that as quality grade improved, (AFT) and (YG) increased while (REA) declined. The percentage of low Choice increased up to 0.6 in. fat and then plateaued at 50%. Also the percentage of YG (4s) increased dramatically as fat-increased beyond 0.6 in. (20% 0.7 in.; 30% 0.75) in. fat. When targeting for 0.4 in. fat, cattle will grade about 46% Choice. If targeting for 50% Choice cattle, will need to be fed to 0.41- 0.45 in fat. Feeding cattle to a máximum of 0.51-0.55 in. maximized quality grade while holding (YG) 4 and 5 discounts to less than 1 percent.

There is a strong negative (-0.73 average for all studies) genetic correlation between marbling and percent lean retail yield (Cundiff, 1987). It means that leaner carcasses tend to have less fat in all major fat depots marbling as well as external, seam and kidney, heart and pelvic (KHP) fat. However an analysis of Angus field data showed there was essentially no genetic correlation between marbling score and external fat thickness (Wilson, 1992).

Bos taurus are significantly more tender than Bos indicus breeds. British breeds are generally tenderer than Continental breeds. In spite of less marbling, cattle carrying the myostatin gene are not different from British in tenderness. This may be a result of dilution of connective tissue due to increased muscle (Cundiff, 1999).

Tenderness is the most important factor contributing to the palatability of beef. Marbling serves as an indicator of tenderness but the relationship is not very strong. A direct objective measure of tenderness via instrumentation would be an important breakthrough that could be help ensure consistent palatability of beef (Harlan et al., 1996).

Implications

These results suggest large differences among breeds for economically important traits. Such information would aid in making decisions for crossbreeding. Highest yield grades can be expected involving crosses Brahman x Angus, Brangus x Brangus, Charolais x Angus, and Hereford x Angus. Reasonable improvement for marbling can be achieved by using inheritance of Hereford x Angus, Brangus, Charolais x Angus, and Hereford x Angus.

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Table 1. Average weights, for productivity traits and marbling scores of individual genetic groups Bos taurus x Bos taurus, Bos indicus x Bos indicus, and Bos taurus x Bos indicus in different proportion.

Genetic group Sire x Dam	FWS lb	HCWT lb	FAT In	KPH %	REA in2	YG	MS a
ChxB	1144	663.52	0.55	2.50	13.40	2.61	330
ChxBr	1166	658.00	0.59	2.50	12.43	2.51	323
H x Br	1003	557.00	0.55	2.60	12.47	2.66	392
H x B	1004	557.92	0.51	2.20	11.98	2.48	332
Ch x A	1045	593.56	0.66	2.70	13.00	2.40	380
B x Br	1041	594.00	0.63	2.10	9.05	3.86	350
Br x Br	1047	580.14	0.59	2.60	12.00	2.05	390
Br x A	1034	596.64	0.70	2.70	11.12	1.75	350
H x A	957	534.00	0.49	2.70	12.27	2.43	400
H x H	942	460.00	0.75	2.80	10.04	3.57	270
B x B	959	559.23	0.71	2.30	8.54	4.17	250

FWS=Final weight at slaughter (lb), HCWT= Hot carcass weight (lb), FAT= Adjusted fat thickness (in), KPH(%)= Kidney-pelvic-heart fat in percentage, REA=Rib eye area (in2), YG= Yield grade, and MS= Marbling score. Chx B=Charolais x Brahman, Ch x Br= FWT = Charolais x Brangus, HxBr=HerefordxBrangus, HxB= Hereford x Brahman, CHxA= Charolais x Angus, BxBr= Brahman x Brangus, BrxBr=Brangusx Brangus, BrxA =Brangus x Angus, HxA=Hereford x Angus, HxH=Holstein xHolstein, and BxB= BrahmanxBrahman

a = 200 to 299 and 300 to 399 corresponded to traces and small amount of marbling within choice quality, respectively.

Table 2. Mean squares for final weight at slaughter, yield grade, and marbling score of crosses involving inheritance Bos taurus x Bos taurus, Bos indicus x Bos indicus and Bos taurus x Bos indicus in different proportion .

Effect	df	FWS	YG	MS a
Sex	1	60912.2 **	13.93**	24,840**
Slaughter Group	2	35054.2 **	0.09	9.7
Sire breed	3	4126.9*	4.97**	8,9
Sires:sire breed	119	1471.8	0.61	8.9
Dams breed	3	15258.3**	3.11**	40.1
Dams:cow breed	119	1201.6	0.43	7.1
Sire x Dam	9	6863.7**	0.47	28.1
Sex x Sire	6			
Sex x Dams	6			
Residual	241	989.7	0,45	710

***Means significant differences (P<.05); **Means highly significant differences (P<.01).**

FWT= Final Weight in kg,; CC= Cold Carcass yield percentage; YG= Yield Grade; DP= Dressing Percentage, a aMS=Marbling Score.

Table 3.Heritabilities (diagonal) and genetic correlations (above diagonal) of carcass traits in crossbred beef cattle.

Trait	FWS	YG	MS
FWS	.45	.43	.45
YG		.47	-.21
MS			.36

FWS= Final weight at slaughter, YG= Yield grade, MS= Marbling

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EFFECTS OF DIETARY AFLATOXIN ON HEPATIC EXPRESSION OF IMMUNE GENES IN GROWING BARROWS

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ABSTRACT: Aflatoxin B1 (AFB1) is produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus* and is found in many grains used as livestock feeds. High dietary levels of AFB1 are associated with impaired liver function and reduced health and performance. The objective of this study was to determine the effects of AFB1 on the hepatic gene expression of growing barrows. Ninety Duroc × Yorkshire crossbred barrows (age = 35 ± 5 d; initial BW = 14.2 ± 3.0 kg) were allocated to 9 pens with 10 pigs per pen, and randomly assigned in a 3 × 3 factorial arrangement of treatments to receive diets containing 0 µg/kg of AFB1 (control), 250 µg/kg of AFB1 (low AFB1), or 500 µg/kg of AFB1 (high AFB1) for 7, 28, or 70 d. Previous RNA sequence analysis revealed 179 transcripts that were highly correlated ($r \geq |0.8|$; $P < 0.001$) with treatment on d 70. Functional analysis revealed 43 unique groups, with one being immune function. Two immune function genes, interleukin 6 (*IL6*) and glutathione peroxidase (*GPX*) and one gene involved in detoxification, epoxide hydrolase (*EH*), were chosen for 1) determination of d 70 gene expression differences using real-time RT-PCR and 2) investigation of d 7 expression levels to determine if these genes could be classified as early responders to AFB1. Relative expression values were tested for treatment effects using the GLM procedure of SAS; least squares means were estimated and tested for pair-wise differences using the Tukey adjustment. Day 70 expression differences were confirmed ($P = 0.066$) for *EH* only; *EH* expression was greater ($P = 0.064$) in control barrows than high AFB1-treated barrows. Day 70 expression of *IL6* and *GPX* did not differ ($P \geq 0.351$) with AFB1 level. Additionally, there were no changes ($P \geq 0.340$) in expression of *EH*, *IL6*, or *GPX* on d 7. Results from this study demonstrate that administration of an AFB1-contaminated diet to growing barrows alters hepatic gene expression, and affects expression of at least one gene in the liver that is associated with hyperalgesia during inflammation and detoxification in the liver. The downregulation of *EH* associated with AFB1 exposure may slow recovery from aflatoxicosis and hence reduce performance.

Key Words: aflatoxin, gene expression, immune function, swine

Introduction

The most potent of the aflatoxins, aflatoxin B1 (AFB1), is produced by the molds *Aspergillus flavus* and *Aspergillus parastiticus* which are found in crops grown under poor environmental conditions (Hussein and Brasel, 2001; Devegowda and Murthy, 2005). Affected crops include but are not limited to corn, oats, barley, cotton seed and copra. Dried distillers grains (DDGS), a source of aflatoxins, are often used as an energy source in ruminant diets. Because aflatoxins produced on corn are not destroyed during ethanol production but instead are concentrated 3-4 fold (FDA, 2006; Wilkinson and Abbas, 2008), DDGS can be a problematic feed source, especially for non-ruminants such as swine as they cannot efficiently metabolize aflatoxins. Administration of AFB1 at ≥ 420 µg/kg for short durations in swine can be detrimental to health and performance (Harvey et al., 1990; Lindeman et al., 1993; Rustemeyer et al., 2010). In addition, AFB1 causes reproductive complications in numerous species (Austin et al., 2012). Aflatoxicosis leads to liver damage; however, susceptibility varies with age, concentration of AFB1 and duration of exposure. The exact mechanisms by which this occurs are unknown. The objectives of this study were to determine the effects of AFB1 on immune gene expression of growing barrows and to identify genes which may act as early responders to AFB1 exposure.

Materials and Methods

Animal Procedures. All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee. Ninety Duroc x Yorkshire crossbred barrows were purchased from a commercial farm at an average age of 35 (± 5) d. Barrows were allocated to 9 pens, with 10 pigs per pen. Each pen was randomly assigned to 1 of 9 dietary treatments: 0 µg/kg of AFB1 (control), 250 µg/kg of AFB1 (low AFB1), or 500µg/kg AFB1 (high AFB1) for 7, 28 or 70 d in a factorial arrangement of treatments. Barrows were allowed to adjust to the new facilities and pen mates for 6 d prior to starting their respective treatments. Barrows were allowed ad libitum access to feed and water throughout the study. Ground corn containing either the low (250 µg/kg) or high (500 µg/kg) level of AFB1 was added to the starter and the grower diets (Rustemeyer et al., 2010). The starter diet was fed from d 0 to 20 and the grower diet was fed from d 21 until the end of the study. Pigs were monitored for signs of

aflatoxicosis including decreased feed intake, average daily gain, body weight, growth rate and listlessness (Harvey et al., 1990; Schell et al., 1993). Barrows were weighed and bled weekly then euthanized on the last day of the trial. Livers were removed, examined for abnormalities and samples snap frozen for RNA analysis. Since previous RNA sequencing data revealed immune regulation as one functionally affected group, two immune genes, interleukin 6 (*IL6*) and glutathione peroxidase (*GPX*), as well as one detoxification associated gene, epoxide hydrolase (*EH*) were analyzed to determine the expression of these genes on d 7 and 70 using real-time RT-PCR.

RNA Extraction. Liver tissue (50-100 mg) was placed in 1ml of TRI reagent (Sigma Chemical Co., St. Louis, MO) and homogenized using an electronic tissue grinder (IKA Laboratories; Wilmington, NC) for 30 sec at maximum speed. The homogenate was allowed to sit at room temperature for 5 min; 0.2 ml of chloroform was added with shaking and the homogenate incubated at room temperature for another 10 min. The homogenate was centrifuged for 15 min at 12,000 g at 4°C. The upper aqueous layer was removed and placed in a new tube containing 0.5 ml of isopropanol, mixed and incubated at room temperature for 10 min. The RNA was precipitated by centrifuging for 10 min at 12,000 g at 4°C. The RNA pellet was re-suspended in 100 µL nuclease-free water and further purified using the Rneasy kit (Qiagen, Santa Clarita, CA). Briefly 350 µL buffer RLT was added to each sample followed by 250 µL absolute ethanol. The samples were mixed and pipetted onto RNeasy columns provided in the kit. Samples were centrifuged and the flow through was discarded. The columns containing RNA were washed with buffer RW1 and centrifuged again. Columns were allowed to incubate for 15 min at room temperature with 80 µL DNase 1 solution (provided in the kit) followed by another wash with RW1. Columns were washed twice with buffer RPE and the RNA was eluted in 100 µL nuclease-free water. The purified RNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Denver, CO) and 2 µg aliquots were placed in 0.5 mL tubes for cDNA synthesis.

cDNA synthesis and Real-Time RT-PCR. Two µg of RNA were mixed with 4 µL reverse transcription buffer (5X) and 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA) in 20 µL total volume. The mixture was placed in a thermocycler for 5 min at 25ºC, 30 min at 42ºC, 5 min at 85ºC and held at 4ºC. The cDNA was diluted with 100 µL nuclease-free water and stored at -20ºC until semi-quantitative real-time PCR was performed. Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) such that amplicons were ~150 bp in size. Real-time RT-PCR was performed by mixing 10 µL of diluted cDNA with 12.5 µL of SYBR© Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), 500 pmol each of the forward and reverse primers, and 0.5µL nuclease-free water in each well of a 96-well plate. Amplification was performed using the IQ5 and 40 cycles of 95ºC for 30 sec and 60ºC for 30 sec. Melting curve analysis was performed post-amplification to ensure the quality of PCR products as noted by the presence of a single peak. Briefly, the PCR plate was heated to 95°C for 3 min and then cooled to 55°C; the temperature was then increased by 0.5°C per sec up to 95°C. Ovine glyceraldehyde 3-phosphate dehydrogenase (*GAP*) was used as the reference gene and all gene expression levels were quantified and reported relative to *GAP* expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Real-time RT-PCR was performed in duplicate for each sample/primer set. Samples analyzed included control (n = 7), low (n = 7) and high (n = 7) liver RNA from d 7 AFB1-treated barrows and control (n = 8), Low (n = 6) and High (n = 8) liver RNA from d 70 AFB1-treated barrows.

Statistical Analysis. The effect of treatment (control versus high AFB1) on relative gene expression levels at d 7 and d 70 was tested using the GLM procedures of SAS; least square means were estimated and tested for pairwise differences.

Gene	Forward Primer	Reverse Primer
GAP	5'gggcatgaaccatgagaagt3'	5'aagcagggatgatgttctgg3'
GPX	5'atgccatcaaatcccaatgt3'	5'cccaattcacggactctgtt3'
EH	5'caagaatggggagatcctga3'	5'gataaacttggggtcggtca3'
IL6	5'atggcagaaaaagacggatg3'	5'gtggtggctttgtctggatt3'

Table 1. Primers used for Real-Time RT-PCR

Results and Discussion

As previously reported by Rustemeyer et al. (2010), average feed intake was less ($P \leq 0.022$) in high AFB1 barrows than in control barrows from wk 5 to 10, and was less ($P < 0.030$) in low AFB1 barrows than in control barrows in wk 5 and again from wk 8 to 10. Also, ADFI was less ($P = 0.022$) in high AFB1 barrows than low AFB1 barrows in wk 10. Decreased ADG ($P \leq 0.014$) was observed in high AFB1 barrows compared to control barrows in wk 8 and 10; no differences ($P = 0.665$) in ADG were noted between control and low AFB1 barrows. Performance differences observed in this study were similar to those reported by Lindemann et al. (1993) in barrows fed 840 µg/kg of AFB1.

Previous RNA sequencing experiments revealed 179 transcripts which were highly correlated with treatment (control, low AFB1, and high AFB1) on d70. Represented within the RNA sequencing data were 43 unique functional groups including one for immune function. Based on these functional groups and a previous report from Yarro et al., (2009), three genes (*GPX, EH, IL6*) were examined using real-time RT-PCR to determine expression differences on d 70 and establish if these genes could be classified as early responders to AFB1 on d 7. Expression levels of *EH* were decreased ($P = 0.064$) on d 70 in high AFB1-treated barrows compared to controls. This was in agreement with Yarro et al. (2009) who found that *EH*, *GPX* and *IL6* were all down regulated in broiler chicks fed 2mg/kg BW. In this study, expression of *IL6* and *GPX* did not differ ($P \geq 0.351$) with AFB1 levels on d 70. Additionally there were no differences in expression of *EH*, *GPX* or *IL6* on d 7 due to AFB1 treatment. Results from this study demonstrate that *EH* is downregulated in response to treatment with

AFB1. Epoxide hydrolase is the major enzyme that degrades epoxy-fatty acids (EFAs). These EFAs are products of the arachidonic acid (ARA) cascade; metabolites of ARA are associated with anti-inflammatory properties. Additionally, *EH* is associated with the detoxification of Aflatoxin-8, 9-epoxide and has been implicated in the alteration of AFB1 hepatocarcinogenesis through inhibition of microsome-generated genotoxicity (Yarro et al., 2009; Guengerich et al., 1996). The decreased expression of *EH* in the high AFB1-treated group may indicate the level of damage to the liver caused by aflatoxin exposure in the high AFB1 group; this may also indicate an altered ability of these barrows to recover from the insult to the liver associated with aflatoxicosis.

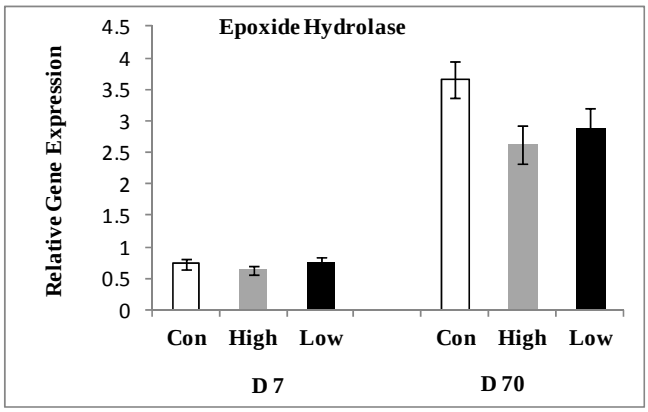

Figure 1. Hepatic expression of *EH* on d 7 and 70 following treatment with 0 µg/kg (con), 250 µg/kg (low) or 500 µg/kg (high) AFB1.

Implications

Aflatoxicosis leads to compromised performance, health and reproductive efficiency in numerous species. Decreased function of immune genes or those that aid in detoxification may affect the ability to recover from insult to the liver and other organs. Identification of affected genes and pathways due to dietary aflatoxins may lead to treatment and prevention strategies for aflatoxicosis. In addition gene expression profiles have the potential to be used to identify animals more tolerant to high dietary levels of aflatoxins.

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REPRODUCTIVE PARAMETERS IN DAIRY CATTLE IN SEMI-ARID CLIMATE

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ABSTRACT. The objective of this study was to evaluate the reproductive performance of a dairy herd management under semi-tech, in a semiarid region, was use the records of 163 cows of different lactations, in four years (2008, 2009 and 2010). The study was conducted of the dairy unit of UJED-FMVZ. There were 115 births, calving was 5.4 % double, 7% abortions. Statistical analysis was by ANOVA, with test of "t" student to tell the difference between half a year, we made a test X for variables measured in % (SPSS, ver. 10). The interval of first service-delivery was 63.0 ± 2.3 days, the first-service conception rate was 33.3, 34.2 y 38.0% during 2008, 2009 and 2010, respectively. In the second service was obtained 65% of pregnancy. Was obtained an average of 2.4 ± 0.13 services per conception. There were 118 ± 6.5 of open days during the evaluated period. Calving interval was 420 ± 9.2 days. We discard 25.2% of adult cows, and we recorded 16.6% a mortality in calves during the first year of life. The problem in milk production is evident. It presise an analysis of more detailed information for each dairy herd, the characterization of the behavior is very important for decision making regarding management practices to be implemented, with the purpose of obtaining acceptable parameters in the production cycle of cows. . .On one hand the reproductive performance of cows is not acceptable think of it of the prolonged period of days open, and it is strongly associated with reduced profitability of cattle, and secondly the high mortality rate of waste that questions the availability of replacement animals.

Key Words: Dairy cattle, Reproductive performance, semi-arid climate.

Introduction

In dairy cattle reproductive efficiency is crucial to assess the profitability of the herd and this is reflected in the ability of the cow to achieve a calving interval of 12-13 months (Chiristie *et al*., 2007; Lilido, 2008) low fertility negatively impacts milk production, increase reproductive management protocols and professional services, discarded as many animals and require more replacements. Calving interval of more to come unless situation being observed in most countries, including developed ones. The pregnancy rate to first service has fallen from 9 to 15% in Japan, Germany, USA, etc. (Lilido, 2008, Nakao, 2008), most of the cows require more than one service to pregnancy (Quintela *et al.*, 2004). The calving interval to ovulation happens 43 days or more, and the number of cows were at 60 pp has increased in recent years (Dobson *et al*., 2007). The pregnancy rate in heifers is 70% to first service, therefore the genetic component or selection of semen does not condition the reduced fertility in cows (De Vries, 2005), situation that may be inherent in the physiological changes during lactation, and the influence of risk factors in each herd and each cow in particular (environment temperature, milk yield, diseases, husbandry, nutrition, etc.). From 1994 to 2003, annual milk production per cow increased 15% in the U.S. and 20% in Mexico (USDA, 2007), where there is growing demand and unmet bovine milk, one way to overcome this deficit is through more efficient livestock breeding behavior (Ramirez and Segura, 2009). The key role of the veterinarian in dairy cattle today, is to identify and understand the factors limiting fertility and implement effective strategies at the lowest cost to improve the reproductive performance of the herd. However, management strategies to maximize reproductive efficiency of cows are multifactorial and differ between different farms (Dalton *et al*., 2006). It should be mentioned that at management under semi-technical and backyard operating conditions one of the main problems is the complete or partial absence of records within herds, making difficult decisions or perform a certain improvement program based on the behavior production and reproduction of each animal. Therefore, the objective of this study was to analyze the information to characterize the reproductive performance of a herd milk producer Semi-technical operating conditions in a semiarid area during three years

Materials and Methods

The work was performed at the dairy farm located in Durango-El Mezquital Km 11.5 to a latitude of 23 ° 59 '12 ", longitude 104 ° 37' 38" and altitude of 1878 meters. Located between parallels 26 º 48 '22" and 22 º 14' 22". The average temperature recorded during 2008-2010 was 25.7 ° C, a maximum and minimum of 31.1 º 5 º C, precipitation of 500 and 700 mm, 50% relative humidity and radiation of 250-270 w/m^2 (CEVAG, 2010). According Köppen climate is BS1 (w) semi-tempered (Aguilar, 2001). We used the records of 115 cows of different numbers of births for 2008 (n = 41), 2009 (n = 39) and 2010 (n = 35). The health and nutritional management of the herd was as follows: provided food concentrate with 21% PC according to milk production (1:4), forage (alfalfa, oats and/or irrigated pasture according to availability and season year) and corn silage. A mineralized salt mixture (12% Ca, 12% P, 1.9% Mg, 11 ppm and is vitamin E 200 IU / kg, including trace minerals) was *ad libitum*. Annually, the animals are immunized against clostridial diseases (*Clostridium spp*) and respiratory viral and bacterial (PI3, IBR, BVD, leptospirosis and *pasteurella spp spp*) and the herd is free of

brucellosis and tuberculosis. Replacement females are vaccinated only once against brucellosis (RB51). Reproductive management was receiving animals: a dry period of 45-70 days, I watch the prepartum period for delivery care and later expulsion of the fetal membranes at 12 h after birth of the calf. The uterine involution was monitored daily the first 20 days postpartum. Estrus was detected twice daily (am-pm) for 30 minutes each time, until the first service record and then return to estrus. Pregnancy diagnosis was performed 45 days after the last service by transrectal palpation. For the writing of the results was used descriptive statistics. The data were analyzed by ANOVA with a student t test was established the difference between means and ran an X^2 test for variables measured in % (SPSS, 2006).

Results and Discussion

The majority of births occurred in May, August and October (14.8, 13.9 and 12.2%) and the lowest, March and June (3.5 and 3.5%). Regarding the incidence of births by age of the year, 17.4% occurred in winter, 22.6% in spring, 31.3% in summer and 28.7% in the fall, concentrating 53.9% during the spring and summer, warmer times and hard for animals. The proportion of male births was higher than that of females (55.3 and 44.7%), birth weight was different between sex ($p < .05$) 40.4 ± .81 and 36 ± .70 for males and females, respectively. Regarding reproductive behavior, parameters per year are shown in Table 1. The incidence of twin births in each year was different ($p < .05$). The overall average births per cow during 2008 to 2010 were 2.3 ± 0.12, birth weight 38.5 ± 0.58 k. The conception rate with 3 or more services was 33.3, 34.2 and 38.0% for 2008, 2009 and 2010, respectively.

Conclusions

The problems in milk production is evident, first the poor reproductive performance of cows which is strongly associated with reduced profitability of herds, and on the other, the high rates of culling of young cows and calf mortality during the nursery stage, which concept raises the cost of replacement animals. Prolonged open days directly affect the culling rate, a good proportion of cows going for infertility. Reproductive efficiency in this type of animal is measured by: the days to first service, open days, calving interval, services per conception, heat detection rate, pregnancy rate and birth rate. The parameters in this case are acceptable, although fertility rates to the first and second service are low. However, it reaches more than 90% with three or more services, hence the average services per conception is greater than that reported by Lilido (2008); Cordova-Izquierdo (2008), Christie *et al*., 2007) of 1.75 to > 3, 1.62 to 2.9 and 1.8 to 1.6. In this case also there was a high percentage of culling and a higher proportion of males at birth, which affects the availability of replacement heifers. De Vries (2005) reported that losses in dairy herds by breeding concept are increasing. Pregnancy rates decreased from 36% to 9%, which resulted in increased open days 112 to 166. The cost for each additional open day varies from $ 3.19 to 5.41 per cow per year. Also reported a loss of 2.4 ± 1.09 k of milk per day open extra, but found that this loss is variable according to the number of lactation. We obtained an average of 119 days open. Cyclicity, fertilization and gestation had a seasonal pattern, the months in which there were higher rates of births were those animals receiving effective service in August, November and January, while those who were served in April , May, June and July gave birth in the months of February, March and June.

Analysis is required for more detailed information of each herd, the characterization of the behavior is very important for making decisions about the management practices to be implemented, in order to have acceptable parameters in the production cycle of cows.

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Table 1. Reproductive parameters in Holstein-Frisian cows per year

Parámeters	**2008**	**2009**	**2010**	**General average**
Number of births	39	41	35	115
Twin births (%)	0a	4.8b	11.4c	5.4
Abortions (%)	7.3 (3/41)	7.8 (3/38)	5.8 (2/34)	7.0
Cull (%)	34.14	21.05	20.5	25.24
*Mortality (%)	19	17.5	13.5	16.6
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
1st service birth interval (d)	64.2 ± 19.4	66.7 ± 5.5	67 ± 4.0	63.0 ± 2.3
Conception 1st service (%)	38.4a	26.8b	31.4	32.2
Conception 2nd service (%)	28.2a	39.02[a]	31.4	32.8
Services by conception	2.3 ± .21a	2.5 ± .24[a]	2.5 ± .26	2.4 ± .13
Open Days (d)	142 ± 13.6a	101 ± 7.7b	113.4 ± 11	118.9 ± 6.5
Calving Interval (d)	439 ± 15.86a	408 ± 10.5[a]	356 ± 8.0	420 ± 9.2

* Birth of calves 0-12 months old
% Statistical difference by X^2
[ab] in rows ($p > 0.05$)

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INDUCTION OF REPRODUCTIVE ACTIVITY IN DAIRY GOATS IN ANOESTRUS IN ARID REGION OF NORTHERN MEXICO

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ABSTRACT. In order to induce reproductive activity during the anoestrus, 50 dairy goats were used in production and of first birth (18 Saanen and 32 French Alpine). The study was conducted on a arid region in goat farm located in Gomez Palacio, Dgo. Mexico. 50 goats were used in production of first birth, with an age of 1.5 years (18 Saanen and 32 French Alpine). .The beginning of treatment, the body condition of the animals was acceptable. Males received 60 days prior to flushing. According to the body weight of the goats, formed three groups: (GI = 19, 25-35 kg), (G II = 18, 36-40 kg) and (G III = 12, 41-50 kg), the which received 200, 300 and 400 IU of PMSG, respectively. The protocol for synchronization of estrus was: sponge with 45 mg of FGA, 265 mcg of cloprostenol sodium and PMSG. Estrus was detected after 12 h post treatment and controlled breeding was done. Statistical analysis was performed using ANOVA and the differences between means were established with a "t" test of student (SPSS, ver 10). The estrous activity beginning at 26.6 ± 5.5 h, with a duration of 25.5 ± 5.5 h. 82% of the goats were in estrus. There was no difference in the length of this presentation and, between race (P> .05). The dose of PMSG had no effect on the length of estrus (p> .05). We obtained a 38% fertility and prolificacy 1.1 and 15.7% of twin births. The length of gestation was 156 ± 3.0 d. Estrus induction was good. The induction of cyclic ovarian activity in goats was good, pregnancy rates were low at first service. However, it requires more efficient use of sires to increase the rate of calving. Use of PMSG in the lowest dose is recommended, due to the animals similar response in presentation, length of estrus and prolificacy.

Key Words: Dairy goat, PMSG, Cloprostenol, Estrous cycle.

Introduction

In many countries estrus synchronization in goats and sheep is a limited practice. The restricted availability of specific drugs leads to the use of products that have been developed and tested in large species (ex. cattle or pigs), so there are not standardized dose protocols for these species and therefore there is also little information regarding the hormonal results protocols for this purpose. The average life of the goat is 7 years and has 3-4 births, which is usually due to the system of herd management. Reproduction of goats is seasonal from late summer to late fall, usually have a birth year, from late winter to late summer, and sexually rest until next season (Ortiz and Mareco, 2007). There are strategies to induce reproductive activity at any time of year, such as the handling of the daylight hours, the male effect or combination of both and estrus synchronization protocols with different hormonal. These practices make possible continuous milk production in herds, as well as the sale of kids in times of convenience for the producer (Heibel, 1990, Alvarez *et al*., 2004). Any of these practices, requires the availability of additional studs, since the libido and quality of semen also lower in non-reproductive periods. The induction and synchronization of oestrus in small ruminants, is for the purpose of manipulating the follicular or luteal phase of the estrous cycle, luteal phase focuses on control interrupt (the corpus luteum regression) or induction of this (providing a source exogenous progesterone) both provide acceptable rates of onset of estrus and fertility. In this kind are common in the protocol which includes using placental gonadotropin treatment complement the base (Wildeus, 2000, Alvarez *et al*., 2004). The aim of this study was to evaluate the reproductive performance of dairy goats anoestrus to estrus synchronization protocol with progestins, prostaglandin and gonadotropin of pregnant mare serum

Materials and Methods

The work was conducted during the months of April to October 2010, in the Ejido Venecia of municipality of Gomez Palacio, Durango, on Highway 22 Km Tlahualilo Gomez Palacio. Geographically it is located at 25 ° 46 '30 "N, 103 ° 19' 31" W, at an altitude of 1,113 meters. The average summer temperature is 37.5 ° C (OEIDRUS, 2011). 50 females were used in production of a birth, with an age of 1.5 years (18 and 32 French Alpine Saanen) in production. To start the treatment, females were chosen with the best fitness and reproductively healthy. They had nine stallions (5 Alpine and 4 Saanen) with an average age of 2.8 years. Prior to the start of the program (60 d), were kept entirely separate females (distance >300 m). Were given an energy supplement 21 days before mating (200 g/d extra feed concentrate with 14% CP) and a tonic and restorative vitaminado one week before mating. After the general assessment, females were subjected to estrus synchronization protocol, after formation of three groups according to body weight. Group I (n = 19) of 25 to 35 k received 200 IU of PMSG, Group II (n = 18) of 36 to 40 k received 300 IU of PMSG, Group III (n = 12) of 41 to 50 k received 400 IU PMSG. Hormonal treatment started with the application of the sponge (day 0), as shown in Figure 1.

After treatment, the stallions were released in turn and race 12, 18 and 24 h post treatment. Controlled service was

provided, served goats were separated, each stallion gave a maximum of six services. Table 2 shows the activities during the experiment. The variables measured were treatment-estrus interval (h), estrus rate (%), duration of estrus (h), effect of race and effect of PMSG dose on the duration of estrus. The data were analyzed using ANOVA and the difference between means was established with a Student t test (SPSS, see 10). For the writing of the results was used descriptive statistics.

Results and Discussion

Live weight and body condition. The average live weight and body condition (BC) was 36.8 ± 5.6 k, minimum 24 and maximum 48 k, and a CC of 2.5 ± 0.34, minimum 2.0 and maximum 3.0 (Figure 2). Weight and BC the start to treatment did not different (P> 0.05) between breeds (Table 1).

Treatment-estrus interval (h). The onset of estrous activity occurred at 24 h after intravaginal device removal and application of PMSG (Table 2). This parameter is not defined; the reports thereon have been treated animals in breeding season, in extensive conditions and meat producers, in which there is variation in the onset of estrus, low hormone treatments similar to that performed in this case. The duration of estrus was similar between races ($p > .05$) 26.7 ± 1.8 and 24.7 ± 1.9 h for Saanen and Alpine French, respectively.

Presentation and length of estrus. The 83.6% (41/49) of the goats showed estrus, which was confirmed visually and with the acceptance to male. A female in estrus were given controlled to start this service. Approximately six hours before the end of the second estrus was provided the second service. The 28% of goats estrous activity beginning at 24 h post-treatment, at 26 h, 52% of the goats were in estrus and estrus 20% started between 36 and 48 h. The onset of estrus by race was similar ($p > .05$) 88.2% and 81.3% for Sannen and Alpina, respectively.

The fertility obtained was 38% (19/50), the prolificacy index was 1.15, there were a 15.7% of twin births and gestation length was 156 ± 3.0 d.

The CC optimum for reproduction in the goat is 3 or 3.5 and is constantly looking to assess since a change of 1 degree, an increase or decrease of 12% of body weight (Kinne, 1995; Manazza, 2006; Gallego and Molina, 1994). Whatever the situation before the loss or gain of the CC in a short period of time, indicates serious problems in managing nutritional or health status of the animal (Giraudo, 2009) determine the cyclicity, fertilization, maintenance of pregnancy, prolificacy, transition period and lactation, hence animals to be subjected to intense reproductive management should maintain a good at optimum DC. Reproductive parameters depend on income from the sale of milk and goats are the producer. Regarding the response of animals to hormonal protocol, Rojero Martinez *et al*., (2008) reported a treatment-estrus interval of 24 h in creole goats under extensive conditions treated with FGA + eCG breeding season; Kausar *et al*., (2009) 65.4 ± 24.0 h in goats receiving 60 mg of MAP and duration of estrus of 29.8 ± 6.7 h and within 21 h to 100% of females in estrus. Rodriguez-Castillo *et al*., (2003) reported a duration and onset of estrus of 35.2 ± 1.4 h and 15.2 ± 1.5 h ($p < .05$), 23.0 ± 3.6 and 24, 4 ± 3.2 h ($p < .05$) for Boer x Nubian goats (in breeding season), under a protocol with FGA + GnRH, FGA + PMSG, PGF_2 + GnRH and PGF_2 α + PMSG. They also found that the duration of estrus is greater when applied in combination with FGA with PMSG and is not affected by the use of PGF_2 α. The onset of estrus is faster with FGA + PMSG *vs* PMSG + GnRH. Use of PGF_2 α delays the onset of estrus. Kausar *et al*., (2009) report that the onset of estrus and duration of 65.4 ± 24.0 h in goats receiving 60 mg of MAP and 29.8 ± 6.7 h in the control group. Regardless of the drug used in the synchronization of estrus, in general, the response of the animals is acceptable in many protocols, so that the same choice should be evaluated from an economic point where addition of the drugs, including labor and handling of the animals. Physiologically, progesterone concentration of 2 to 15 ng/ml in blood, inhibits pulsatile GnRH secretion and LH therefore, preventing follicular maturation and ovulation. During the duration of the sponge in the vagina, there are constant release and absorption of progesterone (or progestin), exogenous PGF_2 α helps to restore pituitary gonadotropin production and the addition of PMSG increases the number of growing follicles and therefore the rate of reproduction in such programs. Fertility indices obtained from goats anestrous are 49 to 53% by natural service, from 33 to 87% with artificial insemination (cervical) and up to 63% with laparoscopy, with results reported using different protocols hormone (Freitas *et al*., 1996; Freitas *et al*., 1997; Ahmed *et al*., 1998, Alvarez *et al*., 2004). In this case, the low fertility contrasts with the high percentage of estrus, which may be due to the stallion, that is to say, lower the ratio female: male in such programs and provide a second service at 24 hours after initiation of estrus this with the purpose of ensuring availability of viable gametes at the time of ovulation. After treatment, estrous activity continued and even goats that were in adjacent pens cyclicity began in late May and June full, which is attributed to an effect of biostimulation. In this case the dose of PMSG had no effect on either the length or the estrus prolificacy rate, but if it affects the cost of the protocol, given the high market price of the gonadotropin. Wildeus (2000), says that besides PMSG expensive, has a positive effect on prolificacy but seriously affects fertility and conception rates by circulating estrogen levels after fertilization.

Conclusions

The induction of cyclic ovarian activity in goats was good; pregnancy rates were low at first service. The availability of stallions for such programs should be increased, and improve the body condition of the animals constantly. Use of PMSG in the lowest dose is recommended, due to the animals similar response in presentation, length of estrus and prolificacy. Hormonal protocols are a viable alternative for obtaining continuous goats milk and also pose no threat to consumer.

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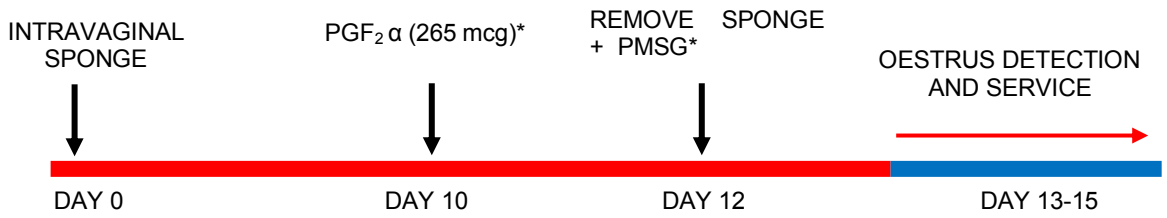

Figure 1. Protocol used for goats in anoestrus (treatments). * Intramuscular Aplication

Table 1. Live weight and body condition (BC) for dairy goat breed at the beginning of estrus synchronization program.

	Race	N	Mean ± STD	SEM
Live weight (k)	1	18	36.15 ± 4.7	1.08
	2	32	37.27 ± 6.1	1.07
BC (1-5)	1	18	2.3 ± 0.31	0.07
	2	32	2.5 ± 0.34	0.06

1 = Saanen 2 = Alpina Francés

Table 2. Estrus in goats treated with PGF_2 α and FGA + PMSG

	No.	Mínimum	Máximum	Mean ± STD	SEM
Post treatment estrus (h)	41	24.00	47.00	26.6 ± 5.5	0.86
Estrus length (h)	41	20.00	31.00	25.5 ± 5.5	0.33

Table 3. Length of estrus in goats treated with PMSG.

Group	Weight (kg)	PMSG (UI)	Long. estrus (h) Mean ± STD	SEM
I (n = 19)	25-35	200	25.4 ± 2.3	0.59
II (n = 18)	36-40	300	25.8 ± 1.2	0.36
III (n = 12)	41-50	400	24.8 ± 2.0	0.62

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ASSOCIATION OF STAT2 SNP GENOTYPES AND GROWTH PHENOTYPES IN HEIFERS FROM AN ANGUS, BRAHMAN AND ROMOSINUANO DIALLEL POPULATION

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ABSTRACT: Components of the growth endocrine axis regulate growth and reproduction traits in cattle. A SNP in the promoter of the signal transducer and activator of transcription 2 (STAT2) has been previously reported to be associated with postpartum rebreeding in a diallel beef population composed of 650 heifers from Angus, Brahman, and Romosinuano breeds. The objective herein was to assess the association between STAT2 SNP (i.e., -rs137066603-A/G-) genotypes and growth traits such as birth weight, weaning weight, and yearling weight in this multi-breed heifer population. This SNP had minor allele frequency > 10% across the three breeds and did not deviate from HardyWeinberg equilibrium (X2 = 1.00, $P > 0.31$). Genotype to phenotype association analyses used a mixed effects model that included phenotype as the response variable, genotype as a fixed term, sire as a random term, and coefficient of ancestry as a covariate. Although these analyses did not reveal any significant association between the STAT2 genotypes and growth phenotypes, the traits birth weight, weaning weight, and yearling weight were associated ($P < 0.05$) with the interaction of SNP genotype and ancestral cluster. A population stratification analysis involving 72 informative SNPs identified the ancestral cluster 1 (inferred primarily from Brahman), and clusters 2 and 3 (inferred primarily from *Bos taurus*). The interaction plots revealed a higher estimated effect of heterozygous genotype in cluster 1 and lower estimates in clusters 2 and 3, as this genotype increased the levels of the traits birth weight (7.4±0.7 kg), weaning weight (69.9±6.4 kg) and yearling weight (72.4±4.5 kg). Like associative analyses with fertility traits, a SNP in the promoter of the STAT2 gene was associated with growth traits in an admixed heifer population composed of Angus, Brahman and Romosinuano breeds. This study suggests a non-additive genetic effect as the heterozygote appeared to be the favorable genotype.

Key Words: heifer, growth traits, SNP genotype.

Introduction

The GH/IGF endocrine axis has been recognized as key regulator of growth and development in animals (Frago and Chowen, 2005). Growth hormone activates its hepatic receptor (GHR) to stimulate secretion of IGF-I through a cell signaling pathway that involves several proteins (Lucy, 2008). Metabolic hormones such as leptin interact with GH/IGF axis to influence development and sexual maturation in heifers (Hegvi et al., 2004).

Members of the STAT family proteins are mediators of both GH and leptin signaling pathways. The *STAT2* locus is on chromosome 5 within a 23 mega-base region that is a QTL associated with growth and body composition in cattle (Rincon et al., 2007).

Brahman x *Bos taurus* crossbreeds are common across the southeastern United States and Mexico as they express greater heterosis than purebreds under sub-tropical environments. Objective herein was to perform a genetic associative study between *STAT2* SNP genotypes and growth traits such as birth weight, weaning weight and yearling weight in an Angus, Brahman and Romosinuano heifer diallel population.

Materials and Methods

Genes within the GH/IGF-I functional axis located in a 23 Mb region of bovine chromosome 5 was identified on the GenMAPP diagram of Farber et al. (2006), and it includes several genes from different physiological groups. These genes were IGF-I, *IGFBP6*, Pro-Melanin Concentrating Hormone (*PMCH*), Signal Transducers and Activators of Transcription 2 and 6 (*STAT2,6*) and Suppressor of Cytokine Signaling 2 (*SOCS2*). Functional regions of each gene were resequenced using DNA from 48 unrelated cattle, in order to increase genetic diversity and improve re-sequencing, following procedures described by Garrett et al. (2008). They were: exons, 1000 bp of the 5'-untranslated region, and the 500 bp of the 3'-untranslated region. Vista alignment (http://pipeline.lbl.gov/) was conducted to identify conserved introns and exons from genes containing multiple exons (> 10). Resequencing was completed at SeqWright (Houston, TX) and provided results of SNP, indel, and microsatellite. Finally, SNP were confirmed with CodonCode aligner (CodonCode® Corporation, Dedham, MA) and then tested to be synonymous or non-synonymous using Polyphen (http://genetics.bwh.harvard.edu/pph), and if they were a tag SNP (Haploview; http://www.broad.mit.edu/mpg).

Animals, Phenotype, and Genotype

Beef heifers from a diallel population (n=650) involving Angus, Brahman, and Romosinuano breeds and their reciprocal crosses were studied. Heifers were born and raised in the Subtropical Agricultural Research Station (USDA-ARS), in Brooksville FL.

Calves were tagged at birth, branded and vaccinated as 2-months old, and weighed at birth, and 205- and 365-d of age. Measures of birth weight, 205-d weight, and 365-d weight were then reported and adjusted by age of calf and age of dam according to Beef Improvement Federation Guidelines. After weaning, heifers were developed by grazing Bermuda and Bahia grass pastures, and they received the reproductive management described by Riley et al. (2010).

Blood samples were collected from each heifer and processed for DNA extraction. The genomic DNA (i.e., 25 ng/µl in TE buffer) was used for genotyping through a Sequenom MassArray platform (GeneSeek, Inc., Lincoln NE). The SNP genotypes were determined using the allelic discrimination procedure. Genotypes were coded AA for homozygous, AG for heterozygous, and GG for opposing homozygous.

Statistics

Statistical analyses were conducted using SAS software (Version 9.2; SAS Inst. Inc., Cary, NC), which included Genetic Analysis Tools of SAS (Saxton, 2004).

Simple Statistics and Frequencies

Simple descriptive statistics for continuous growth traits (i.e.., birth weight, weaning weight, and yearling weight), were calculated using PROC MEANS. Assumptions of normality of data distribution and equality of variances were tested using PROC UNIVARIATE Allele and genotype frequencies, as well as deviation from Hardy-Weinberg equilibrium, were estimated with PROC ALLELE.

Population Stratification Analyses

The STRUCTURE software was used to determine genetic structure and correct for population stratification (Pritchard et al., 2007) because an admixed population was involved in this study. This program used a Bayesian approach to assign individual animals to inferred ancestral subpopulations on the basis of their allele frequencies at multiple loci, and estimated the individual's proportion of membership or admixture, termed Ancestry Coefficient.

Association of Genotype to Phenotype

Association analyses for growth continuous traits were conducted using PROC MIXED. Only polymorphisms with genotype frequencies greater than 10% (e.g., minor allele frequency (MAF) > 10%) were considered appropriate to be included in this associative study. The statistical model for genotype to phenotype association was:

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

y_{ijklmn} = phenotypic value of trait,

μ = population mean,

A_i = fixed effect of SNP genotype,

B_j = fixed effect of year of birth (i.e., 2002, 2003, 2004, and 2005),

C_k = fixed effect of age of dam (i.e., 2, 3, 4, 5 to 10, or 11 yr and older; BIF, 2006);

D_l = covariate of coefficient of ancestry (i.e., admixture proportion from inferred Brahman cluster),

E_m = covariate of ordinal birth date of the heifer,

F_n= random effect of sire using the *Z* statistic to test if Ho:$\sigma_w^2 = 0$, and

e_{ijklmn} = random residual error.

Association analyses were also conducted including primary ancestral clusters and their interaction with genotype as a fixed effect instead of the covariate, coefficient of ancestry. The interaction was visualized with Proc GPLOT.

If genotype was found to be a significant ($P < 0.05$) source of variation in association analyses for growth traits, then PDIFF option was executed to generate preplanned pair-wise comparison of least square means for genotype, which included Bonferroni adjustment.

Results and Discussion

Only one SNP in the promoter of the STAT2 gene had MAF > 10% across the three breeds. This SNP did not deviate from Hardy-Weinberg equilibrium ($X^2 = 1.00$, $P > 0.31$). Population stratification analysis was executed with 72 SNP which were useful as ancestral informative markers (i.e., minor allele frequency < 10%), as they provided information relative to ancestral differences in allele frequencies (i.e., coefficient of admixture or ancestry). Results for these analyses suggested a unique ancestry in Brahman heifers (cluster 1), and a common ancestry among heifers from Angus (cluster 2) and Romosinuano (cluster 3) breeds, as indicated in table 1.

Simple statistics of the traits evaluated in this study are depicted in table 2. Year of birth ($P < 0.01$), coefficient of ancestry ($P < 0.05$) and sire ($P < 0.05$) were significant sources of variation in prediction of growth traits. Mixed model analyses including coefficient of ancestry as covariate did not reveal any significant association between the STAT2 genotypes and growth phenotypes. However, the traits birth weight, weaning weight, and yearling weight were associated ($P < 0.05$) with the interaction of SNP genotype and ancestral cluster. Mixed model analyses of these traits involving the interaction of cluster and genotype revealed a higher estimated effect of heterozygous genotype in cluster 1 (inferred primarily from Brahman) and lower estimates in clusters 2 and 3 (inferred primarily from *Bos taurus* breeds). Sliced interaction plotting (Figure 1) revealed that the heterozygous genotype increased the levels of the traits birth weight (7.4 ± 0.7 kg), weaning weight (69.9 ± 6.4 kg) and yearling weight (72.4 ± 4.5 kg)..

The STAT proteins are important intracellular second messengers of GH and leptin, which are involved in heifer development and puberty (Fruhbeck, 2006). Growth hormone stimulates hepatic secretion of IGF-I, and this endocrine axis (i.e., GH/IGF) is highly involved in growth and adiposity in cattle (Rhoads et al., 2008).

Interferons, prolactin and placental lactogen, which are associated to pregnancy and fetal development in cattle, also induces a signaling pathway based on the phosphorylation of STAT proteins (Maj and Chelmonska, 2007). In a previous study, the same *STAT2* polymorphism was found to be associated with the traits days to calving and calving interval in beef heifers from a diallel population through a non-additive effect (Luna-Nevarez et al., 2011).

Like associative analyses with fertility traits, a SNP in the promoter of the *STAT2* gene was associated with growth traits in an admixed heifer population composed of Angus, Brahman and Romosinuano breeds. This study suggests a non-additive genetic effect as the heterozygote appeared to be the favorable genotype.

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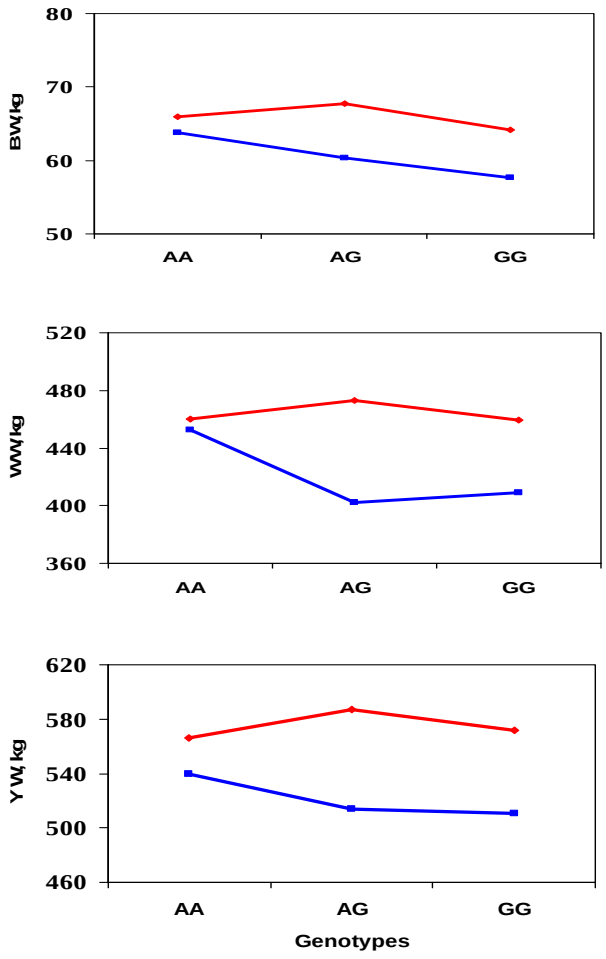

Figure 1. Graphic representation of the interaction among SNP genotypes in *STAT2* (e.g., AA, AG and GG) and ancestral clusters for the traits birth weight (BW), weaning weight (WW) and yearling weight (YW; $P < 0.05$). Clusters from *Bos indicus* (1; red line) and *Bos taurus* (2 and 3; blue line) breeds are represented.

Table 1. Ancestry proportions of Angus, Brahman and Romosinuano diallel breed groups in clusters outputted from STRUCTURE.

		Ancestry Clusters		
Breed Group	N	1	2	3
Angus	66	0.012	0.694	0.294
Brahman	59	0.878	0.029	0.082
Romosinuano	129	0.040	0.461	0.498
Angus x Brahman	116	0.462	0.334	0.204
Angus x Romosinuano	136	0.039	0.497	0.464
Brahman x Romosinuano	143	0.474	0.234	0.292

Table 2. Number of observations, and mean ± SE for birth weight, weaning weight, and yearling weight, in a diallel population of Angus, Brahman and Romosinuano heifers.

Breed Group	N	Growth Traits[1]		
		Birth Wt (kg)	Weaning Wt (kg)	Yearling Wt (kg)
Angus	66	29.1 ± 0.4	171.9 ± 2.1	237.9 ± 4.0
Brahman	59	30.2 ± 0.5	200.2 ± 2.3	252.2 ± 2.9
Romosinuano	129	29.7 ± 0.2	176.7 ± 1.8	222.4 ± 2.5
Angus x Brahman	116	33.1 ± 0.3	208.1 ± 2.4	281.0 ± 2.7
Angus x Romosinuano	136	30.8 ± 0.3	187.3 ± 1.8	249.2 ± 2.2
Brahman x Romosinuano	143	33.0 ± 0.4	207.0 ± 1.8	265.2 ± 2.3

[1]Traits adjusted according to Beef Improvement Federation guidelines (2006).

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PHENOTYPIC DIFFERENCES FOR GROWTH, BODY COMPOSITION, AND FEED EFFICIENCY AMONG ANGUS, HEREFORD, AND RED ANGUS BULLS GROUPED ON RESIDUAL FEED INTAKE[1]

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ABSTRACT: Phenotypic differences for growth, body composition and feed efficiency among Angus, Hereford, and Red Angus bulls grouped on residual feed intake were determined using data collected at a central bull test station in Yerington, NV. Individual DMI were collected for bulls over a four year period (Angus, n=186; Hereford, n=66; Red Angus, n=38) using the GrowSafe® feeding system. Feed conversion ratio (DMI/ADG; FCR), partial efficiency of growth (ADG/DMI for growth; PEG), and residual feed intake (RFI), computed as the difference between actual DMI and DMI predicted by the linear regression of DMI on ADG and mid-test metabolic body weight (MMWT), were calculated for each bull within pen contemporary group. Bulls were subsequently divided into low (<-0.5 SD; n=88; RFI=-0.902 kg/d), marginal (±0.5 SD; n=117; RFI=0.055 kg/d), and high (>0.5 SD; n=84; RFI=0.918 kg/d) groups. RFI group means were analyzed using ANOVA, including the fixed effects of RFI group, breed and year. No interactions were detected (P>0.05). No significant differences existed among RFI groups for 205-d adjusted weight (P=0.50), 365-d adjusted weight (P=0.90), ADG (P=0.49), Kleiber ratio (P=0.76), frame score (P=0.13), scrotal circumference (P=0.08), pelvic area (P=0.55), ultrasound ribeye area (P=0.06), and ultrasound percent intramuscular fat (P=0.13). Significant RFI group differences were detected for FCR, PEG, DMI, RFI and ultrasound back fat (P<0.01). Low RFI grouped bulls appeared to have a more favorable FCR (5.93 kg) and PEG (0.38) with reduced DMI (10.88 kg/d) and less back fat (6.94 mm). There were no significant differences among Herefords, Red Angus, and Angus for RFI (P=0.95). Herefords appeared to have more favorable DMI (11.05 kg/d; P<0.01) and PEG (0.33; P=0.02). Results suggest that phenotypic selection of bulls with low RFI can be used to improve efficiency of growth without adversely impacting growth, reproductive measures, and body composition.

Key Words: Beef Cattle, Body Composition, Feed Efficiency, Growth Traits

Introduction

Beef cattle production profitability depends upon efficient use of feed inputs. At an estimated 50% or more of the total cost (Kennedy et al., 1993), feed inputs account for the largest percent of beef production costs (Arthur et al., 2001; Herd et al., 2003). While past and current practices in livestock genetic selection to improve beef production have primarily focused on output traits, there is a renewed interest in economically relevant traits (Golden et al., 2000) that represent the input side of production. These include, among others, feed efficiency measures.

Over two dozen measures of feed efficiency have been employed as selection tools, yet they are not without adverse effects, including an associated increase in mature cow size. One of the most familiar is feed conversion ratio (FCR). The traditional approach of placing selection pressure on growth and FCR may result in increased mature cowherd size, requiring greater inputs for cowherd maintenance (Herd and Bishop, 2000; Archer et al., 1999). A more desirable selection tool to improve beef production efficiency is one that has a favorable or negligible impact on other growth and carcass traits.

Residual feed intake (RFI; Koch et al., 1963) is an alternate measure of feed efficiency. RFI is the difference between an animal's actual intake and its predicted intake, accounting for average daily gain (ADG) and body weight maintenance measured as mid-test metabolic body weight for bulls on test.

RFI is reported to have favorable or negligible phenotypic and genetic relationships with feed intake, FCR, and body weight (Arthur et al., 2001; Hoque et al., 2006; Tedeschi et al., 2006). Furthermore, Carstens et al. (2002) and Nkrumah et al. (2007) reported high RFI steers had greater amounts of ultrasound back fat compared to low RFI steers. Wang et al. (2012) reported no significant differences in reproductive performance and fertility among beef bulls categorized as inefficient or efficient based on RFI.

Characterizing efficient cattle and understanding the relationships between RFI and production traits are keys to a successful beef cattle selection program that manages the efficient use of production inputs while optimizing output. Thus, the objective of the this study was to determine phenotypic differences for growth, body composition and feed efficiency among Angus, Hereford, and Red Angus bulls grouped on residual feed intake.

Materials and Methods

Individual bull DMI data were collected over four years at a commercial central bull test station in Yerington, NV. In 2007, individual feed intake data were collected over a 62-d period on 47 Angus, 25 Hereford, and 10 Red Angus bulls. In 2008, individual feed intake data were collected on 46 Angus, 13 Hereford, and 6 Red Angus

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bulls over a 72-d test period. In 2009, individual feed intake data were collected on 32 Angus, 18 Hereford, and 9 Red Angus bulls over a 73-d test period. Data for years 2010 and 2011 were omitted to due lack of complete records, and in 2012, individual feed intake data were collected on 61 Angus, 10 Hereford, and 13 Red Angus bulls over a 70-d test period. During all test years, bulls received a grower ration after a 28-d adjustment period at the start of each test followed by a 7-d transition period to a finisher ration. The grower ration averaged 12% CP, 13.96% CF, 3.73% fat, 1.45 Mcal/kg NEm, and 0.86 Mcal NEg on a dry matter basis. The finisher ration was comprised of 10.76% CP, 5.53% CF, 4.03% fat, 1.81 Mcal/kg NEm, and 1.15 Mcal/kg NEg on a dry matter basis. Average on-test and off-test weights were collected each year. Mid-test metabolic body weight (MMWT) was calculated as the mid-test $BW^{.75}$. Individual DMI data were collected during the test period using the GrowSafe® automated feeding system (Grow Safe Systems Ltd., Airdrie, Alberta, Canada). A certified ultrasound technician using an Aloka 500 real-time unit equipped with a 3.5-MHz transducer collected all ultrasound data. A certified veterinarian determined scrotal circumference and pelvic area measurements each year during breeding soundness evaluation (BSE).

Four different measures of feed efficiency were calculated for each bull. Feed conversion ratio (FCR) was measured as the ratio of DMI to ADG. Partial efficiency of growth (PEG) was computed as the ratio of ADG to DMI for growth (Koch et al., 1963). The Kleiber ratio was calculated as the ratio of ADG to mid-test metabolic body weight (Bergh et al., 1992; Arthur et al., 2001a). Residual feed intake (RFI) as defined by Koch et al. (1963) was computed for each bull within pen contemporary group as the difference between actual DMI and DMI predicted by the linear regression of DMI on ADG and MMWT. Based upon calculated RFI, animals were pooled and then divided into low (<-0.5 SD; n=88; RFI=-0.902 kg/d), marginal (±0.5 SD; n=117; RFI=0.055 kg/d), and high (>0.5 SD; n=84; RFI=0.918 kg/d) groups (Basarab et al., 2003).

RFI group means were analyzed using ANOVA (Statistix9, 2008), including the fixed effects of RFI group, breed, year and all interactions. All pairwise comparisons were made using Tukey HSD (Statistix9, 2008).

Results and Discussion

No significant interactions between RFI grouping with breed or year were detected. RFI groups did not significantly differ in 205-d adj. weaning weight (P=0.50), 365-d yearling weight (P=0.90), frame score (P=0.13), and ADG (P=0.49), suggesting that phenotypic selection for reduced RFI would have minimal impact on growth (Table 1). Nkrumah et al. (2007) and Lancaster et al. (2009) reported similar trends for ADG and final BW among RFI grouped bulls.

RFI grouped bulls did not appear to differ in scrotal circumference (P=0.08) and pelvic area (P=0.55). It appears that selection for improved RFI will not negatively impact the reproductive characteristics of bulls on test. Similar results for scrotal circumference were reported by Wang et al. (2012). Bulls categorized in inefficient and efficient groups based on RFI did not differ in BSE traits. While not examined in this study, Wang et al. (2012) did report the number of bulls failing to meet the 60% minimum sperm motility requirement tended to be greater in the efficient bull group; however, in a multisire natural mating experiment, the average progeny number per sire was greater for the efficient bull group. Selection for improved feed efficiency, specifically using RFI, and its impacts relative to bull fertility warrants more investigation.

No significant RFI group differences were detected in percent intramuscular fat (uIMf, P=0.13). Similar trends in uIMF were reported by Lancaster et al. (2009). Marginal RFI grouped bulls tended to have larger ultrasound ribeye area (89.13 cm^2, P=0.06) compared to low (86.64 cm^2) and high (85.95 cm^2) RFI grouped bulls. In comparison, Nkrumah et al. (2007) and Lancaster et al. (2009) reported no differences in ultrasound ribeye area among RFI groups.

Differences in ultrasound back fat (uFT) were detected between the low RFI grouped bulls and those in the marginal and high groups. Results of the current study are in agreement with previous work regarding ultrasound body composition traits. Previous research suggests that low RFI bulls tend to have lower uFT compared to high RFI bulls (Carstens et al., 2002; Nkrumah et al., 2007). Robinson and Oddy (2004) reported that selection for improved RFI would likely result in lower subcutaneous fat, a result supported by Lancaster et al. (2009) who reported RFI appeared to be correlated with 12th rib fat thickness. Nkrumah et al. (2004) further noted that while efficient bulls tended to be leaner, carcasses had adequate back fat to prevent being downgraded. The inclusion of uFT as a measure of body composition in the linear regression model to predict DMI removed uFT differences between RFI bull groups (Berryhill et al., 2009). Inclusion of uFT in the model accounted for more variation in DMI as reported by Basarab et al. (2003). Lancaster et al. (2009) reported minimal reranking of growing animals compared to a linear regression only including ADG and MMWT. The relationship between body composition and RFI in finishing cattle suggests the inclusion of body composition in the calculation of RFI may be warranted.

No significant differences existed among RFI group for Kleiber ratio (P=0.76). Similar results were reported by Nkrumah et al. (2004) and Crowley et al. (2010). Favorable RFI group differences were detected for FCR, PEG, DMI and RFI (Table 1; P<0.05). Low RFI grouped bulls appeared to have a more favorable FCR (5.93 kg) and PEG (0.38) with reduced DMI (10.86 kg/d). This is an advantage in FCR of 1.04 kg of feed less/kg of gain compared to the high RFI group. Furthermore, low RFI bulls appeared to consume 1.46 kg of DM less per day compared to high RFI bulls. The gain in efficiency and reduced feed intake were achieved without detectable differences in ADG and body weight. Lancaster et al. (2009) reported similar findings, citing a 15% reduction in feed intake between low and high RFI bulls, despite no

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detection of differences in ADG and body weight as found in the current study. Low RFI bulls had higher PEG (0.38; P<.05) compared to high RFI bulls (0.28). Results of the current study are in agreement with Nkrumah et al. (2004) and Nkrumah et al. (2007). Selection for improved RFI should result in improvements in production efficiency and PEG.

No apparent differences in FCR (P=0.51), Kleiber Ratio (P=0.67), and RFI (P=0.95) were detected among Angus, Herefords, and Red Angus; however, in this study, Herefords appeared to have more favorable PEG (0.33) compared to Angus (.32; P<0.05). Similarly, Herefords appeared to have lower DMI (11.05 kg/d; P<0.05) compared to both Angus (12.11 kg/d) and Red Angus (11.75 kg/d). Unlike the current study, Crowley et al. (2010) reported a significant difference in RFI and Kleiber ratio among Irish performance-tested Angus and Hereford bulls. More research investigating breed differences for efficiency measures, specifically RFI, is warranted.

Implications

Selection tools with relevance to production efficiency have significant economic implications for commercial cattlemen. Results suggest that phenotypic selection of bulls for improved RFI can be used to improve efficiency of growth without adversely impacting growth, frame size, reproductive measures, and body composition. Differences detected between RFI groups for uFT may need addressing in future evaluations of RFI. Furthermore, more research is necessary to characterize breed differences for residual feed intake.

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Table 1. Least square mean estimates (SE) for phenotypic performance for bulls classified as low (<-0.5 SD; n=88; RFI=-0.902), marginal (±0.5 SD; n=117; RFI= 0.055 kg/d)), or high (>0.5 SD; n=84; RFI=0.918 kg/d) based upon individual residual feed intake (RFI) values.

	RFI Group		
Trait	Low	Marginal	High
205-d Adj. Weaning Wt. (kg)	311.58 (3.60)	313.50 (3.05)	307.77 (15.67)
365-d Yearling Wt. (kg)	521.79 (14.09)	518.33 (14.53)	525.78 (6.21)
Average Daily Gain (kg/d)	1.87 (0.03)	1.86 (0.03)	1.82 (0.04)
Feed Conversion Ratio (kg feed/kg gain)	5.93 (0.11)[a]	6.43 (0.09)[b]	6.97 (0.11)[c]
Partial Efficiency of Growth (ADG/DMI for growth)	0.38 (0.006)[a]	0.31 (0.005)[b]	0.28 (0.006)[c]
Kleiber Ratio (ADG/mid-test metabolic body weight)	0.02 (0.0004)	0.02 (0.0004)	0.02 (0.0005)
Frame Score	6.30 (0.09)	6.12 (0.07)	6.07 (0.09)
Scrotal Circumference (cm)	35.13 (0.39)	36.16 (0.30)	35.63 (0.39)
Pelvic area (cm^2)	144.39 (1.67)	145.42 (1.42)	142.95 (1.75)
DMI (kg/d)	10.86 (0.12)[a]	11.71 (0.10)[b]	12.32 (0.12)[c]
RFI (kg)	-0.90 (0.05)[a]	0.07 (0.04)[b]	0.92 (0.05)[c]

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

Table 2. Least square mean estimates (SE) for ultrasound body composition for bulls classified as low (<-0.5 SD; n=88; RFI=-0.902), marginal (±0.5 SD; n=117; RFI= 0.055 kg/d)), or high (>0.5 SD; n=84; RFI=0.918 kg/d) based upon individual residual feed intake (RFI) values.

	RFI Group		
Characteristic	Low	Marginal	High
Ultrasound Ribeye Area (cm^2)	86.64 (1.09)	89.13 (0.92)	85.95 (1.14)
Ultrasound Back Fat (mm)	6.94 (0.24)[a]	7.68 (0.20)[b]	7.95 (0.31)[b]
Ultrasound Percent Intramuscular Fat	4.46 (0.12)	4.15 (0.10)	4.36 (0.13)

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

Table 3. Least square mean estimates (SE) for phenotypic feed efficiency for Angus, Hereford, and Red Angus bulls.

	Breed		
Trait	Angus (n=186)	Hereford (n=66)	Red Angus (n=38)
Feed Conversion Ratio (kg feed/kg gain)	6.48 (0.06)	6.34 (0.10)	6.50 (0.14)
Partial Efficiency of Growth (ADG/DMI for growth)	0.316 (0.003)[a]	0.333 (0.005)[b]	0.318 (0.007)[ab]
Kleiber Ratio (ADG/mid-test metabolic body weight)	0.02 (0.0002)	0.02 (0.0004)	0.02 (0.0006)
DMI (kg/d)	12.11 (0.07)[a]	11.05 (0.11)[b]	11.75 (0.12)[a]
RFI (kg)	0.03 (0.03)	0.02 (0.05)	0.04 (0.06)

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

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CRITERIA FOR OPTIMUM FEEDLOT HARVEST ENDPOINT

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ABSRACT: One of the most important management questions for finishing cattle is "How long to feed them to maximize profit?" My objective was to develop extension tools to answer this question. In the case of an owner independent of the feedlot, the profit maximizing strategy is to feed the pen of cattle until the costs for that day exceed the pen's gain in value, that is, the marginal net revenue becomes negative. In the case of a feedlot owning the cattle, and recognizing that the feedlot's profit maximizing objective is to make money on the pens over time, then the economic principle is to feed cattle until their marginal net revenue (daily increase in value minus daily cost) no longer exceeds the average daily net revenue for an average animal in a pen that would replace the current one. However, complex interaction between type of cattle, market demand and price, ownership of the feedlot and/or the cattle, and application of marketing and management tools make profit prediction difficult. To address this, the beef cattle simulation module in UC software TAURUS is employed to dynamically predict animal value depending on carcass weight, quality, possible defects and other market factors, and efficiency of gain. This method integrates the biology of the animal, its management, and market prices to project animal and carcass characteristics through time, and associated costs and potential revenue. Results show an interaction between frame size and optimal feeding period exists, with larger frame cattle benefiting from earlier feedlot entry, or smaller frame cattle benefiting by being grown on forage diets or pastures before feedlot entry. If the feedlot does an exceptionally good job of buying cattle low and selling them high, then the average daily revenue is high, and cattle are fed shorter times. Also, if average daily net revenue is projected to decrease in the coming months after a set of cattle may be sold, cattle should be fed longer.
Key Words: Beef Cattle, Feedlot Profit, Mathematical Models

Introduction

As feedlots are used to finish more cattle, improved strategies evolve. One of the most important management questions once cattle are in the feedlot is "How long to feed them to maximize profit?" However, complex interaction between type of cattle, market demand and price, ownership of the feedlot and/or the cattle, and application of marketing and management tools make profit prediction difficult. Addressing these interactions requires proper application of economic principles, identifying the relevant biology of the animals being fed, and tools such as bioeconomic models to estimate animal performance. When used properly to answer the appropriate question, these tools provide insight into improved management of feedlot cattle.

Economic Considerations

One of the basic principles of microeconomics is profit maximization. However, for the feedlot case one needs to determine who is trying to maximize profit on a pen of cattle—the owner of the cattle or the feedlot. If the feedlot also owns the cattle, there are different constraints and objectives than if the cattle are owned, or partly owned by another person. Also, whether a pen of cattle can be replaced with another, as opposed to one pen per year or time period, makes a difference.

In the case of an owner independent of the feedlot, and assuming this person's capital is not limited by a particular pen of cattle, the profit maximizing strategy is to feed the pen of cattle until the costs for that day exceed the pen's gain in value, that is, the marginal net revenue becomes negative. Hence, the cattle are fed until the feed and other costs for the last day exceed that day's cattle gain multiplied by the cattle's value per unit weight. Special attention is warranted in this scenario with discounts in cattle price for increasing carcass weight or decreasing yields, as this decrease in the pen's value may be sudden. For this reason the more variable the cattle in a particular pen, the shorter is the optimum feeding period for profit maximization (Smith et al., 1988).

In the case of a feedlot owning the cattle, and recognizing that the feedlot's profit maximizing objective is to make money on the pens over time, not just for any particular pen of cattle, then the economic principle is to feed cattle until their marginal net revenue (daily increase in value minus daily cost) no longer exceeds the average daily net revenue for an average animal in a pen in the feedlot. Average daily net revenue is the profit for an animal divided by the number of days that animal was in the feedlot. As long as the average net revenue is positive (the feedlot is making a profit on the cattle, as well as on the feedlot enterprise), cattle owned by the feedlot will be fed fewer days than those owned by others. Again, as in the case above, more variable cattle will be fed fewer days than more uniform ones, but this is less important in the feedlot owning the cattle scenario since the shorter days on feed reduce the chance of discounts.

There are two exceptions or alternatives in the above scenarios. If the cattle owner cannot feed additional cattle until a pen of cattle is sold, then the objective is profit maximization over time, not for a pen of particular pen of cattle. Therefore, their cattle should be fed as in the case of the feedlot owning the cattle above. If the feedlot owns the

cattle, and for some reason cannot use the pen again after the cattle are sold/removed (often in case where only one set of cattle are fed in each physical pen annually), then their profit maximization objective is as first scenario above where the cattle are owned independently of the feedlot—indeed the cattle profit is then independent of the feedlot profit.

The above depends on the marginal net revenues as cattle progress in a feeding period. Note that in the early days after a pen of cattle is put on feed, their total value is less than the money invested and the early feed and processing costs (which would result in negative returns if sold at that point). However, the marginal net revenue is usually positive—the value of their daily gain exceeds the daily feed cost. Hence profits are increasing, or losses decreasing. If this is not the case, and marginal returns are negative, prolonging the feeding period increases the loss, and the cattle should be sold. This is not unusual for chronically sick individuals. Even for well animals, a pen of cattle can loose money if fed to the proper endpoint—it is just that they will loose less money if fed to that endpoint.

Marketing is a major consideration—that is the relative difference between the price paid for cattle and that for which they are sold. In the case of an independent owner, it is often the difference between profit and loss, independent of the discussion above on proper time of marketing the animals. For the feedlot owning the cattle (or the capital limited outside owner), it is more interesting. Since the optimal feeding strategy is to feed until marginal net revenue decreases to average daily revenue for typical pens in the feedlot, the average daily revenue is important—and depends more on the difference between the prices paid and received for cattle. If the feedlot does an exceptionally good job of buying cattle low and selling them high, then the average daily revenue is high, and cattle are fed shorter times. In fact, if it is quite high days on feed approaches zero, and the feedlot simply becomes a holding pen for cattle being transferred in ownership—a cattle broker's location. Understanding how a feedlot's average daily net revenue may change over time is thus important to optimizing profit for cattle owned by that feedlot. Thus if average daily net revenue is projected to decrease in the coming months after a set of cattle may be sold (incoming cattle prices too high, market cattle prices declining, feed prices increasing), the argument is to feed the cattle longer.

Biological Constraints

The above economic discussion seems to ignore the resulting animal's product and its growth. However, this is not the case because the animal's value is quite dynamic, depending on carcass weight, quality, possible defects and other market factors; its profit also depends on efficiency of gain. These biological parameters are complex and have been the focus of much beef cattle research for over fifty years. Rather than summarize all the literature, an overview of the major factors affecting how animal value changes as feedlot animals approach slaughter endpoints follows.

Carcass weight is a major driver of revenue, and animal value increases in direct proportion unless other factors interact to decrease value per unit carcass weight. Thus, in most analysis, feeding animals to heavier weights usually increases profit. Constraints due to excessive carcass size in slaughter plants, or undesirably large muscle cuts limit carcass size by decreasing value of the carcass. Increasing cost of gain as animals age also constrain carcass size, usually as a result of an increasing proportion of the feed being used for animal maintenance (related to body weight) instead of gain. Hyer et al. (1986) also showed that as steers reached or exceeded normal market weight, feed intake decreased, further exacerbating the above effect of less feed available for gain.

Carcass weight and the yield of retail cuts in the carcass change with increasing body weight, and result in value differences as well. Pricing cattle on a live weight basis requires consideration of the relative increase in carcass weight as a proportion of live weight. But pricing cattle on either a live weight or carcass weight basis must also consider the decrease in retail cut yield as carcass fatness increases. As animals finish in a feedlot fatness increases, so beef yield as a proportion of carcass decreases, particularly so for genetically fatter animals. In the US this is called Yield Grade, and steep discounts for animal with higher Yield Grades effectively limit time on feed.

Although Yield Grades, or carcass yield, become less desirable with time on feed, carcass quality, Quality Grade in the US, generally improve. Genetics and feeding strategy affect carcass quality with certain breeds (and sires) exhibiting greater marbling and other improved meat qualities. Steroid status of the animal often affects marbling, and interacts with age the animal enters the feedlot. Aggressive anabolic implant use earlier in life seems to decrease marbling; the younger the animal is when entering the feedlot enhances marbling. This probably depends on the endpoint at which marbling is measured—calves are often fed longer before slaughter at a lighter weight than yearling or older cattle. Backfat of calves reaches a given level at a lighter body weight than for older cattle, so they are often slaughtered younger and lighter, to avoid carcass yield discounts and possibly resulting in decreased total value due to lighter carcasses. Yearlings may be more profitable if the cost of gain is high, and the trend to feed these older cattle increases with feed and grain prices.

An interesting interaction between frame size (mature weight of the animal) and optimal feeding period exists, with larger frame cattle benefitting from earlier feedlot entry, or smaller frame cattle benefitting by being grown on forage diets or pastures before feedlot entry. On forage diets, backfat does not increase with body weight as it does on feedlot rations (Sainz et al., 1995), thus the smaller frame animal can be fed to larger, more profitable weights after a period of restricted growth on a lower energy diet.

The NRC (2000) accounts for this using an equivalent weight concept, the weights at which different animals reaches 28% body fat. Thus, one makes an adjustment on body weight to account for different frame size and management effects.

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EFFECTS OF TRANSPORT DURATION PRIOR TO FEEDLOT PLACEMENT ON PERFORMANCE OF PRECONDITIONED BEEF CALVES DURING RECEIVING AND FINISHING

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ABSTRACT: Angus × Hereford calves (n = 428) 2 locations were blocked by sex, stratified by age and BW, and assigned randomly to 1 of 3 transport durations [4 h (**4H**), 8 h (**8H**), or 12 h (**12H**)] following commingling at an auction market and before feedlot placement. Calves were weaned at 183 ± 17 d of age, penned according to treatment (n = 5 pens·treatment^{-1}·location^{-1}), and fed a common diet (16.7% CP, 1.07 Mcal NE_g/kg) during a 30-d preconditioning period. At weaning, calves were weighed, vaccinated against respiratory and clostridial pathogens, and treated for internal and external parasites. Booster vaccinations were administered 14 d later. Preconditioning ADG, final BW, and incidence of undifferentiated fever were not different ($P \geq 0.21$) among treatments. After preconditioning, calves were transported 4 h to an auction market where they were commingled for 12 h. Calves within each treatment were then loaded onto a motor carrier and subjected to assigned transport durations from the auction market to a feedlot. Calves were weighed before shipment to the auction facility and at feedlot arrival. Calves were then penned according to treatment and sex (n = 3 pens·treatment^{-1}·sex^{-1}) and fed a common receiving diet (16.8% CP, 1.07 Mcal NE_g/kg) for 57 d. Feedlot arrival BW were similar ($P = 0.44$) among treatments; however, transport shrink was greater ($P < 0.01$) for 8H and 12H calves than 4H calves. Receiving ADG and G:F of 8H and 12H calves were improved ($P \leq 0.05$) compared to 4H calves. Conversely, DMI was not different ($P = 0.85$) among treatments. Overall incidence of undifferentiated fever during receiving was modest (< 5%) and was not different ($P = 0.67$) among treatments. Subsequently, heifers were removed from the trial and steers (n = 276; 4 pens·treatment^{-1}) were transitioned to a finishing diet (13.8% CP, 1.26 Mcal NE_g/kg) over a period of 14 d. Final BW, finishing ADG, and DOF were not different ($P \geq 0.34$) among treatments. Similarly, carcass weight, carcass traits, lung condition, and liver condition were also not different ($P \geq 0.10$) among treatments. Under the conditions of our study, performance of preconditioned beef calves during receiving and finishing was not influenced negatively by transport durations of up to 12 h immediately following exposure to auction market conditions and prior to feedlot placement.

Keywords: health, preconditioning, transport

Introduction

Transport stress is indicated as a predisposing factor for BRD upon feedlot arrival (Grandin, 1997; Taylor et al., 2010); moreover, body weight lost during transport may be recovered slowly during receiving because of poor DMI (Coffey et al., 2001). Previous research examining the effects of transport stress compared performance of preconditioned and unpreconditioned calves subject to a single transport duration (Pritchard and Mendez, 1990). Additionally, research focused on effects of transport duration on calf performance dealt with non-preconditioned calves transported for > 20 h (Cole et al., 1988).

Thus, our goal was to evaluate the ability of preconditioned calves to perform in the feedlot after being transported for time periods typical for cattle that originate from and are fed in the Great Plains. Our specific objective was to measure performance during receiving and finishing of preconditioned beef calves subject to transport lengths of 4 to 12 h from an auction market to a feedlot.

Materials and Methods

Animal care practices used in this study were approved by the Kansas State University Animal Care and Use Committee (protocol no. 2978.1).

Angus × Hereford calves (n = 428; initial BW = 210 ± 33 kg) originating from the Kansas State University commercial cow-calf herds in Manhattan, KS and Hays, KS were used in this experiment. Calves were weaned at 183 ± 17 d of age. All calves were de-horned and steer calves were castrated before 60 d of age.

At the time of maternal separation, calves were weighed individually and given initial vaccinations against respiratory pathogens (Bovi-Shield Gold® 5, Pfizer Animal Health, Exton, PA), clostridial pathogens (Ultrabac® 7, Pfizer Animal Health, Exton, PA), and *H. somnus* (Somubac®, Pfizer Animal Health, Exton, PA). In addition, all calves were treated for internal and external parasites (Ivomec®, Merial Limited, Atlanta, GA). Booster vaccinations were administered 14 d later.

Following initial processing, calves were confined to 1 of 15 pens within each location (minimum area = 200 m^2/calf; bunk space = 0.46 m/calf; 5 pens·treatment^{-1}·location^{-1}). Calves were fed a diet formulated to

promote 1 kg ADG at a DMI of 2.5% of BW during the weaning phase of the study (Table 1).

Table 1. Composition of the weaning diet

Ingredient composition	DM, %
Alfalfa extender pellets	33.9
Corn gluten feed	18.7
Wheat middlings	14.8
Cracked corn	10.9
Cottonseed hulls	8.1
Dried distillers grain	11.9
Supplement*	3.7
Nutrient composition	**Amount**
Crude protein, % of DM	16.7
NE_m, Mcal/kg	1.69
NE_g, Mcal/kg	1.07

* Supplement contained Vitamin A, limestone, molasses, salt, Zn sulfate and Rumensin® 90.

All calves were monitored for symptoms of respiratory disease at 0700 and 1400 daily during the preconditioning phase of our study. Calves with clinical signs of BRD, as judged by animal caretakers, were removed from pens and evaluated. Calves were assigned a clinical score (scale: 1 to 4; 1 = normal, 4 = moribund), they were weighed, and assessed for fever. Calves with a clinical illness score > 1 and a rectal temperature > 40.0°C were treated with therapeutic antibiotics according to label directions (1st incidence = Baytril®, Bayer Animal Health, Shawnee Mission, KS; 2nd incidence = Nuflor®, Merck Animal Health, Summit, NJ). Cattle were evaluated 72 h post-treatment and retreated based on observed clinical signs.

After 28-d of preconditioning on respective ranches of origin, calves from each location were transported 4 h to a commercial auction market and commingled for 12 h on the premises. Following commingling, calves were loaded by treatment aboard 3 separate motor carriers and subjected to a transport duration of either 4 (4H), 8 (8H), or 12 (12H) h from the auction market to the Western Kansas Agricultural Research Center Feedlot in Hays, KS.

Table 2. Composition of the receiving diet

Ingredient composition	DM, %
Ground sorghum grain	50.4
Wet distillers grains	11.6
Ground sorghum hay	35.9
Supplement*	2.1
Nutrient composition	**Amount**
Crude protein, % of DM	16.82
NE_m, Mcal/kg	1.69
NE_g, Mcal/kg	1.08

* Supplement also contained Rumensin®90, Tylan®, ammonium sulfate, Ca, and Na.

Upon arrival at the feedlot, calves were stratified by sex and penned according to treatment (3 pens·sex^{-1}·treatment^{-1}; minimum area = 200 m^2/calf; bunk space = 0.46 m/calf). Calves were fed a single diet during a 57-d receiving phase and daily DMI was recorded (Table 2). Animals were fed once daily at 0700 h and bunks were evaluated each morning at 0630 h. If the feed allowance from the previous day was consumed, the subsequent feeding was increased by approximately 2% of the previous feed delivery. Bunks were managed using a slick-bunk management method to minimize feed refusals (Pritchard and Burns, 2003). Calf health was monitored as during the weaning phase of the study. Clinical illnesses were treated in the same manner as during the ranch-of-origin weaning period. Individual BW was recorded after 29 and 57 d on feed.

Following receiving, a subset of heifers (n = 152) was removed from the trial for herd replacements. The remaining heifers and all steers (n = 276; 4 pens·treatment^{-1}) were adapted to a finishing diet over a period of 21 d (Table 3). Steers were implanted with Component TE-IS (Elanco Animal Health) on d 1 of finishing and reimplanted with Component TE-S (Elanco Animal Health) 90 d later.

Table 3. Composition of the finishing diet

Ingredient composition	DM, %
Ground sorghum grain	73.2
Wet distillers grains	11.7
Sorghum silage	12.1
Supplement*	3.0
Nutrient composition	**Amount**
CP, % of DM	13.8
NEm, Mcal/kg	1.88
NEg, Mcal/kg	1.26

* Supplement contained Rumensin® 80, Tylan® 40, limestone, salt, and trace minerals.

After 120 d on feed, calves were scanned by ultrasound using an Aloka 500V (Aloka Co., Ltd, Wallingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-125 mm window) to estimate subcutaneous fat thickness over the 12th rib. Steers were assigned to 1 of 3 harvest dates based on this scan to meet an average carcass endpoint of 11.5mm of fat depth over the 12th rib. Final live BW of calves was measured within 48 h of harvest.

Calves were transported approximately 3 h to a commercial abattoir on their respective harvest date. At the abattoir, lungs were examined for lesions as described by Bryant et al. (1996) and livers were examined for presence of abscesses according to procedures described by Brink et al. (1990). Carcasses were chilled for 48 h; subsequently, they were ribbed and graded. Carcass measurements were collected by digital imaging software and included 12th-rib fat thickness, 12th-rib loin eye area, and marbling score. Using these measurements, yield grade and quality grade were assigned according to USDA (1997). Kidney-pelvic-heart fat was determined by difference in carcass weight after removal of all internal fat by dissection.

Preconditioning performance, receiving intake, receiving performance, finishing performance, and carcass characteristics were analyzed as a completely randomized design with pen as the experimental unit (PROC MIXED; SAS Inst. Inc., Cary, NC). No treatment*sex ($P \geq 0.05$) or treatment*location ($P \geq 0.05$) interactions detected, thus sex and location were removed from the final analysis. Pen within treatment was used as the random term. Incidence of sickness during preconditioning and receiving was analyzed using PROC GLIMMIX (SAS Inst. Inc., Cary, NC). All models included terms for treatment, sex, and location. Pen within treatment was used as the random effect. When protected by a significant F-test ($P < 0.05$), least squares treatment means were separated using the method of Least Significant Difference. Treatment differences were considered significant at $P < 0.05$.

Results and Discussion

There were no treatment differences in BW ($P = 0.97$; Table 4) or ADG ($P = 0.21$) at the end of the preconditioning period. This was expected as all calves were managed in the same manner before application of the transportation treatments. Incidence of undifferentiated fever during preconditioning was not different among treatments ($P = 0.67$) and modest overall (< 7% across all treatments).

Transport shrink was calculated as the difference between BW measured upon arrival at the feedlot and BW measured 24 h before transport to the auction facility. Calves transported for 4 h lost less ($P < 0.01$) BW than calves transported 8 or 12 h (Table 5). Transport shrink was not different ($P = 0.27$) among calves transported 8 or 12 h.

In beef cattle, shrink increases as the length of transport increases (Knowles, 1999), with the majority of BW losses occurring within the first 4 h (Coffey, et al., 2001). Calves of similar BW to those used in our study (< 271 kg) had greater feedlot morbidity after transport-induced shrink exceeded 2.6% of pre-transport BW (Cernicchiaro et al., 2012a and 2012b). By that standard, calves assigned to all treatments in our study were at relatively high risk for BRD (BW shrink = 2.9 to 5.2 %) and poor growth performance during receiving.

Data describing the effects of preconditioning on transport shrink is equivocal. Pritchard and Mendez (1990) noted differences in shrink between preconditioned calves and non-preconditioned calves transported for the same length of time; however, treatment effects were inconsistent between experiments. Conversely, Barnes et al. (1990) reported that calves preconditioned for 22 d before auction market commingling and transport had less BW loss than calves weaned and transported to the auction market the same day. During receiving, ADG of 8H and 12H calves was greater ($P < 0.01$) than that of 4H calves (Table 5). There were no treatment differences ($P = 0.85$) in DMI as the receiving DMI was limited to 2.5% of BW/d. Conversely, G:F by 8H and 12H calves was improved ($P \leq 0.05$)compared to 4H calves. Favorable ADG and improved G:F by calves transported 8 or 12 h during receiving may be explained by the replenishment of gut fill lost during transport. Self and Gay (1972) reported that calves transported ~1000 km lost 8.3% of pre-transport BW during transit and needed 12 d to recover lost BW. In our study, there were no treatment differences ($P = 0.88$) in BW at the end of receiving. Consequently, we concluded that increased shrink in the 8H and 12H calves had little or no long-term effect on calf performance.

We subjected calves to transport conditions that, according to previous reports, could have increased susceptibility to BRD and other diseases. Cattle from the central region of the US that were transported > 250 km had greater risk for BRD morbidity and mortality upon arrival at the feedlot than cattle transported < 250 km (Cernicchiaro et al., 2012a). In contrast, we observed no treatment differences ($P = 0.67$) in incidence of undifferentiated fever during receiving, possibly because overall incidence of fever during receiving was small. Arguably, the major benefit of preconditioning is separating temporally the occurrence of stressors such as castration, dehorning, weaning, vaccination, transport, and marketing (Cole, 1985). Calves in our study did not seem to manifest any lasting effects of transport on health and performance during receiving, possibly because these stressful events were separated in time.

Finishing ADG did not differ (P = 0.92) among treatments. No treatment differences ($P = 0.88$) were observed in pre-harvest BW among treatments (Table 6). Cole et al. (1985) summarized 8 trials where ADG of preconditioned and non-preconditioned calves were compared during finishing; they reported no differences between groups. Similarly, we noted no differences ($P = 0.34$) in days on feed, despite feeding to a common physiological end-point (Table 7). Hot carcass weights were not different ($P = 0.50$) among treatments. Measures of carcass fat content, such as marbling score and USDA yield grade, were also not different ($P \geq 0.25$) among treatments. We also evaluated lungs and livers at the packing plant, as they can provide valuable evidence about subclinical disease incidence during finishing; however, frequency of lung lesions and liver abscesses were not different ($P \geq 0.10$) among treatments.

Implications

We interpreted these data to suggest that feedlot performance and health of beef calves preconditioned under as described in our study were not impacted negatively by auction-market commingling or transport of up to 12 h prior to feedlot placement.

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Table 4. Pre-shipment performance of preconditioned beef calves transported for 4, 8, or 12 h after auction-market commingling and prior to feedlot arrival.

	Transport duration, h				
	4	8	12	SEM	*P*-value
Initial BW, kg	211	210	210	4.0	0.91
Final BW*, kg	223	224	224	3.8	0.97
ADG, kg/d	0.41	0.50	0.49	0.052	0.21
Incidence of undifferentiated fever, %	4.27	6.84	4.63	3.136	0.68

* Final BW was measured 24 h prior to application of transport treatments

Table 5. Receiving performance of preconditioned beef calves transported 4, 8, or 12 h after auction-market commingling and prior to feedlot arrival.

	Transport duration, h				
	4	8	12	SEM	*P*-value
Initial BW*, kg	216	213	212	3.6	0.44
Transport shrink†, % of initial BW	2.91[a]	4.81[b]	5.15[b]	0.301	< 0.01
BW at d 29, kg	253	252	252	4.7	0.95
BW at d 57, kg	284	286	284	4.2	0.88
ADG, kg/d					
Arrival to d 29	1.28	1.36	1.37	0.086	0.50
Arrival to d 57	1.18[a]	1.28[b]	1.26[b]	0.029	< 0.01
DMI, kg/d	7.50	7.50	7.50	0.003	0.85
G:F	0.157[a]	0.171[b]	0.168[b]	0.0050	0.05
Incidence of undifferentiated fever, %	2.05	3.08	1.43	1.818	0.67

* Initial BW was measured at feedlot arrival.
† Calculated as the difference between final BW (Table 4) and initial BW at feedlot arrival (Table 5) following transport treatments.
[a, b] Means within rows without common superscripts differ ($P < 0.05$)

Table 6. Finishing performance of preconditioned beef calves transported 4, 8, or 12 h after auction-market commingling and prior to feedlot arrival.

	Transport duration, h				
Item	4	8	12	SEM	*P*-value
Initial BW*, kg	284	286	284	4.2	0.88
Harvest BW†, kg	563	559	558	10.7	0.89
ADG, kg	1.78	1.79	1.79	0.038	0.93
Days on feed	167	161	161	4.4	0.34

* BW at the end of the 57-d receiving period.
† BW measured 24 h before harvest.

Table 7. Carcass characteristics of beef calves transported 4, 8, or 12 h after auction-market commingling and prior to feedlot arrival.

	Transport duration, h				
Item	4	8	12	SEM	*P*-value
Hot carcass weight, kg	342	341	335	6.2	0.50
Marbling score	44.1	44.5	45.7	1.47	0.52
USDA yield grade	3.13	3.29	3.02	0.153	0.25
12^{th}-rib fat thickness, mm	11.1	11.4	10.1	0.81	0.27
Longissimus area, cm^2	27.8	26.9	27.3	0.50	0.31
KPH, %	2.69	2.74	2.69	0.099	0.84
Calves with ≥ 1 liver abscess, %	15.3	34.6	25.4	8.13	0.10
Calves with ≥ 1 lung lesion, %	44.4	38.5	37.3	8.99	0.68

[a] Marbling score: 30 = $Slight^{00}$, 40 = $Small^{00}$, 50 = $Modest^{00}$; ex. 55 = $Modest^{50}$.

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WEANING METHOD INFLUENCES FINISHING PERFORMANCE WITHOUT IMPACTING CARCASS CHARACTERISTICS

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ABSTRACT: We evaluated finishing performance of beef steers (n = 234) that had previously been subject to 1 of 3 ranch-of-origin weaning methods 28 d in duration: drylot weaning + dam separation (**D**), pasture weaning + fence-line contact with dams (**PF**), and pasture weaning + fence-line contact with dams + supplemental feed delivered in a bunk (**PF+S**). Steers assigned to D were fed a preconditioning diet designed to promote 1- kg ADG at a DMI of 2.5% of BW (17.7% CP and 0.93 Mcal NE_g/kg); PF steers had access to native forage only; and PF+S steers had access to native forage and received a ration of the diet fed to D at a rate of 1% of BW 3× weekly. After preconditioning, steers were transported to a feedlot, penned by treatment (n = 3 pens·treatment^{-1}) and fed a common receiving diet for 57 d. Subsequently, steers were transitioned to a finishing diet over 21 d and fed to a common endpoint (12.7 mm 12th-rib fat thickness). At the beginning of finishing, steers assigned to D were heavier ($P < 0.01$) than steers assigned to PF or PF+S. Conversely, Steers assigned to PF had greater finishing ADG ($P < 0.01$) than D or PF+S. There were no differences ($P = 0.14$) in DOF among treatments, despite PF steers weighing 24 kg less ($P < 0.01$) at the start of the finishing phase. No differences were detected ($P = 0.40$) in finishing period DMI. Gain:feed was greater ($P < 0.01$) in PF steers than either D or PF+S steers. Body weight at harvest did not differ ($P = 0.56$) among treatments. We noted no differences ($P = 0.49$) in hot carcass weight among treatments; moreover, 12th-rib fat thickness, KPH, marbling score, USDA yield grade, and longissimus muscle area were unaffected ($P \geq 0.36$) by treatments. We interpreted these data to suggest that, under the conditions of our study, steers preconditioned on pasture without supplementation were able to compensate for previous nutrient restriction during finishing.

Keywords: beef cattle, finishing, preconditioning

Introduction

Calf weight gains during preconditioning are variable and may be affected by weaning method (Bailey et al., 2012). Decreased weight gain during preconditioning may carry over into the finishing phase and influence performance and carcass characteristics. Previous research demonstrated that modest weight gains during preconditioning resulted in reduced calf body weights throughout a 10 wk growing period following preconditioning, relative to calves fed more aggressively during preconditioning (Price et al., 2003). In contrast, Mathis et al. (2008) found that calves preconditioned on native range weighed less at the end of a 45 d preconditioning period and gained more weight during the first 75 d of finishing than calves preconditioned in a drylot. Mathis et al. (2009) compared low- and high-input pasture preconditioning methods and found no difference in finishing performance or profitability of the calves from weaning through harvest. Thus, a producer who retains ownership of calves through finishing may be able to employ a low-cost preconditioning program to minimize costs, while expecting similar finishing performance relative to a high-cost preconditioning program.

Our objectives were to measure growth and health among beef steers during finishing that had previously been subject to 1 of 3 ranch-of-origin preconditioning programs: 1) drylot preconditioning + dam separation, 2) pasture preconditioning + fence-line contact with dams, and 3) pasture preconditioning + fence-line contact with dams + supplemental feed delivered in a bunk.

Materials and Methods

Animal care practices used in our study were approved by the Kansas State University Animal Care and Use Committee (protocol no. 2978.1).

Angus × Hereford steers (n = 234; initial BW = 228 ± 34 kg) originating from the Kansas State University commercial cow-calf herds in Manhattan, KS and Hays, KS were used in this experiment. Steers were 180 ± 19 days of age at weaning. All steers were de-horned and castrated prior to 60 d of age. At weaning, steers were weighed individually and assigned randomly to 1 of 3 ranch-of-origin weaning methods: drylot weaning + dam separation (**D**), pasture weaning + fence-line contact with dams (**PF**), and pasture weaning + fence-line contact with dams + supplemental feed delivered in a bunk (**PF+S**).

All steers were individually weighed and given initial vaccinations against respiratory pathogens at maternal separation (Bovi-Shield Gold® 5, Pfizer Animal Health, Exton, PA), clostridial pathogens (Ultrabac® 7, Pfizer Animal Health, Exton, PA), and *H. somnus* (Somubac®,

Pfizer Animal Health, Exton, PA). In addition, all steers were treated for internal and external parasites (Ivomec®, Merial Limited, Atlanta, GA). Booster vaccinations were administered 14 d later.

Within location, steers assigned to PF and PF+S were maintained for 28 d in a single native pasture (minimum area = 48 ha). Dams were maintained for the first 7 d of this period in adjacent native pastures that afforded fence-line contact with their steers (minimum frontage = 200 m; 4-strand, barbed-wire fence with the bottom 2 wires electrified). Fresh water, salt, and mineral supplements were available continually. Steers assigned to D were transported (< 48 km) immediately after separation from dams and confined within location to a single earth-surfaced pen (minimum area = 200 m^2/calf; bunk space = 0.46 m/calf).

Steers assigned to D were fed a diet (17.7% CP and 0.93 Mcal NE_g/kg) formulated to promote 1 kg ADG at a DMI of 2.5% of BW during the weaning phase of the study. Steers assigned to PF had access to native forage only, whereas steers assigned to PF+S steers had access to native forage and received a supplement of the diet fed to D at a rate of 1% of BW 3× weekly. No adjustments were made to feed delivery rate during the weaning phase. Steers assigned to PF+S were sorted into a single pen located adjacent to the fence line shared with dams at 0900 on Mondays, Wednesdays, and Fridays during the weaning phase. The ration was offered in portable bunks (bunk space = 0.46 m/calf). Pens afforded drinking water in open-topped tanks and consumption of the ration was complete by 1100 at each feeding episode.

All steers were monitored for symptoms of respiratory disease at 0700 and 1400 daily during the weaning phase of our study. Steers with clinical signs of BRD, as judged by animal caretakers, were removed from pens or pastures and evaluated. Steers were assigned a clinical illness score (scale: 1 to 4; 1 = normal, 4 = moribund), were weighed and assessed for fever. Steers with a clinical illness score > 1 and a rectal temperature > 40.0°C were treated with therapeutic antibiotics according to label directions (1st incidence = Baytril®, Bayer Animal Health, Shawnee Mission, KS; 2nd incidence = Nuflor®, Merck Animal Health, Summit, NJ). Cattle were evaluated 72 h post-treatment and retreated based on clinical signs.

At the end of the 28-d weaning period, all steers were transported 4 h from their respective ranch of origin to the Western Kansas Agricultural Research Center in Hays, KS and weighed individually upon arrival. Steers were then stratified by sex and assigned to 1 of 18 pens by treatment (6 pens·treatment^{-1}). Animals were fed once daily (14.9% CP and 0.93 Mcal NE_g/kg) at 0700 h and bunks were evaluated each morning at 0630 h. If the previous days feed was consumed, total feed delivered was increased by approximately 2% of the previous days feed delivery. Bunks were managed using a slick-bunk management method to minimize feed refusals (Pritchard and Bruns, 2003). Dry matter intake was estimated based on feed delivered to the pen. Calf health was monitored as during the weaning phase of the study.

After a 56-d receiving period, steers were adapted to a finishing ration over a period of 21 d (Table 1). Steers were implanted with Component TE-IS (Elanco Animal Health) on d 1 of finishing. Feeding management during finishing was identical to that previously described for the receiving period.

After 120 d on feed, steers were scanned by ultrasound using an Aloka 500V (Aloka Co., Ltd, Wallingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-125 mm window) to determine subcutaneous fat thickness over the 12th rib. Steers were assigned to 1 of 3 harvest dates based on this scan to meet an average carcass endpoint of 11.5 mm of fat depth over the 12th rib in each harvest group. Final live body weights were collected within 48 hours of harvest.

Steers were transported approximately 3 h to a commercial abattoir on their respective harvest dates. At the abattoir, lungs were examined for lesions as described by Bryant et al. (1996) and livers were examined for presence of abscesses according to procedures described by Brink et al. (1990). Carcasses were chilled for 48 h; subsequently, ribbed and graded. Carcass measurements were collected by digital imaging software and included 12th-rib fat thickness, 12th-rib loin eye area, and marbling score. Using these measurements, yield grade and quality grade were assigned according to USDA (1997). Kidney-pelvic-heart fat was determined by difference in carcass weight after removal of all internal fat by dissection.

Finishing performance and carcass characteristics were analyzed as a completely randomized design with pen as the experimental unit (PROC MIXED; SAS Inst. Inc., Cary, NC). All models included terms for treatment, and location. No location * treatment interactions were detected ($P \geq 0.05$), thus location was removed from final analysis. Pen within treatment was used as the random term. When protected by a significant F-test ($P \leq 0.05$), least squares treatment means were separated using the method of Least Significant Difference. Treatment differences were discussed when $P \leq 0.05$; tendencies were discussed when $P > 0.05$ and ≤ 0.10.

Results and Discussion

Finishing Performance. The BW difference ($P < 0.01$; Table 2) between drylot-weaned steers and un-supplemented pasture-weaned steers was 24 kg at the beginning of finishing. Unsupplemented pasture-weaned steers gained BW at a greater ($P = 0.01$) rate and had greater ($P < 0.01$) G:F during finishing than D steers or PF+S. Similarly, Mathis et al. (2008) reported that pasture-weaned steers had greater finishing ADG than drylot-weaned steers through the first 75 d on feed but, over the entire finishing period, there were no differences in ADG. In contrast, Kumar et al. (2012) noted no differences in finishing ADG or G:F of steers that had been subject to 1 of 3 forage-based backgrounding programs that induced initial feedlot BW differences similar to ours.

The key difference between our study and others heretofore cited is that our pasture-weaned steers lost BW

during preconditioning (-0.3 kg/d; Bailey et al., 2012); moreover BW differences persisted through a 57-d receiving period. Our steers that were preconditioned in a drylot were observed at the feed bunk more often during the first 6 d of receiving than steers assigned to either pasture-preconditioned treatment; moreover, drylot steers had greater ADG during receiving (Bailey et al., 2012). Additionally, we observed no differences (P = 0.40) in finishing DMI among treatments. Under the conditions of our experiment, the PF+S steers compensated fully for previous nutritional restriction during the finishing period.

The number of days on feed was not different (P = 0.14) among treatments. Similarly, BW at harvest was not different (P = 0.56) among treatments, likely because harvest occurred at a predetermined physiological endpoint based on backfat thickness. Mathis et al. (2009) also did not note differences in final BW between steers preconditioned at either a high or low rate of gain.

Carcass Characteristics. Hot carcass weight was not different (P = 0.49) among treatments (Table 3). Yield grade, marbling score, and 12th-rib fat thickness did not differ ($P \geq 0.38$) among treatments. Based hot carcass weight and harvest determination methodology (i.e., common backfat thickness endpoint), it appeared that the nutritional restrictions that PF steers were subject to during preconditioning did not alter carcass quality. Other researchers (Hersom et al., 2004; Sharman et al., 2010) reported that the type of growing program employed prior to finishing had minimal effects on marbling score when treatments were fed to a common 12th-rib fat thickness endpoint. In the aforementioned studies, BW differences at the end of the growing period were ~100 kg; our BW difference at the beginning of finishing was only 24 kg and may not have been large enough to affect carcass characteristics.

Implications

Unsupplemented pasture-weaned steers weighed less at the beginning of finishing than drylot-weaned steers but gained BW at a greater rate during finishing. We interpreted these data to suggest that preconditioned steers compensated during the finishing period for previous nutritional restriction. There were no differences in days on feed when, fed to a common degree of 12th-rib fat. Low-input preconditioning programs that involve pasture weaning may not have negative impacts on finishing performance or carcass characteristics of beef cattle under the conditions of our study and may be a means of reducing costs associated with preconditioning.

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Table 1. Composition of the finishing diet

Ingredient composition	DM, %
Ground sorghum grain	74.3
Wet distillers grains	11.8
Sorghum silage	12.3
Supplement*	1.5

Nutrient composition†	Amount
CP, % of DM	12.7
NE_m, Mcal/kg	1.87
NE_g, Mcal/kg	1.21

* Supplement contained Rumensin® 80, Tylan® 40, limestone, salt, and trace minerals

†Calculated using the values of the NRC (2000)

Table 2. Finishing performance of beef steers subjected to 1 of 3 ranch-of-origin preconditioning regimens

Item	D	PF	PF + S	SEM	*P*-value
Initial BW, kg	316[a]	292[b]	297[b]	4.3	< 0.01
Harvest BW, kg	561	570	559	7.9	0.56
ADG, kg	1.60[a]	1.79[b]	1.65[a]	0.038	< 0.01
DMI, kg/d	10.72	10.72	10.73	0.012	0.40
Gain:Feed	0.150[a]	0.167 [b]	0.153[a]	0.0034	< 0.01
Days on feed	163	169	164	2.7	0.14

[a, b] Means within rows without common superscripts differ ($P < 0.05$)

D = Drylot; PF = Pasture-weaned; PF+S = Pasture-weaned + supplement

Table 3. Carcass characteristics of beef steers subjected to 1 of 3 ranch-of-origin preconditioning regimens

Item	D	PF	PF + S	SEM	*P*-value
Hot carcass weight, kg	349	343	346	4.9	0.49
Marbling score	49.4	50.6	50.7	3.54	0.92
USDA yield grade	3.5	3.3	3.3	0.16	0.38
12^{th}-rib fat thickness, mm	12.8	11.6	12.3	0.93	0.42
Longissimus area, cm^2	79.0	78.4	80.2	1.19	0.36
KPH, %	2.65	2.43	2.57	0.148	0.37

[a] Marbling score: 30 = $Slight^{00}$, 40 = $Small^{00}$, 50 = $Modest^{00}$; ex. 55 = $Modest^{50}$

D = Drylot; PF = Pasture-weaned; PF+S = Pasture-weaned + supplement

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EFFICIENCY OF EARLY-WEANED BEEF CALVES IS NOT IMPROVED BY RESTRICTING FEED INTAKE DURING AN 84-d GROWING PERIOD

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ABSTRACT: Harvested forages become scarce and expensive during times of drought; moreover, grains typically have lesser unit cost of energy than forages. Our objective was to evaluate the performance and efficiency of light-weight, early-weaned beef calves program fed a dry-rolled sorghum-based diet (15.6% CP, 1.20 Mcal NE_g/kg) with intake levels adjusted to achieve 1 of 3 rates of gain during an 84-d post-weaning growing period: 1) 0.45 kg ADG (**LOGAIN**; 1.5% of BW DMI), 2) 0.91 kg ADG (**MIDGAIN**; 2.0% of BW DMI), and 3) 1.36 kg ADG (**HIGAIN**; 2.5% of BW DMI). Angus × Hereford calves (n = 243; initial BW = 156 ± 31 kg; average age = 113 ± 17 d) were stratified by sex and assigned randomly to treatment (n = 3 pens·treatment^{-1}·sex^{-1}). Daily feed allowances were estimated based on initial BW; feed deliveries were adjusted to meet targeted gains every 28 d based on BW at the end of the preceding period. Carcass characteristics (i.e., 12th-rib fat thickness, longissimus muscle depth, and marbling score) were evaluated ultrasonically at the end of the 84-d growing period. Incidence of undifferentiated fever was not different (P = 0.95) among treatments. Average daily gain increased (P < 0.01) as feed allowance increased; HIGAIN calves were heavier (P < 0.01) than either MIDGAIN or LOGAIN calves at the end of the 84-d experiment. Conversely, targeted ADG were not achieved (0.55, 0.69, and 0.88 kg for LOGAIN, MIDGAIN, and HIGAIN, respectively). Per design of our study, DMI was greater (P < 0.01) for HIGAIN (4.32 kg/hd/d) calves than for MIDGAIN (3.48 kg/hd/d) calves; moreover, DMI of MIDGAIN calves was greater (P < 0.01) than that of LOGAIN (2.65 kg/hd/d) calves. Gain efficiency did not differ (P = 0.83) among treatments. Fat thickness over the 12th rib was greater ($P \leq$ 0.02) for HIGAIN than either LOGAIN or MIDGAIN calves. In contrast, there were no differences (P = 0.14) in marbling between treatments. Longissimus muscle depth was less ($P \leq$ 0.04) in LOGAIN calves than in MIDGAIN or HIGAIN calves. Under the conditions of this experiment, feed efficiency of early-weaned beef calves was not improved by restricting DMI of a concentrate-based diet during an 84-d growing phase.

Keywords: beef calves, early weaning, intake restriction

Introduction

Early weaning can be used by cow-calf producers to reduce rangeland stocking rates by 20 to 30% during periods of drought (Rasby, 2007). Early-weaned calves can weigh less per day of age than calves weaned at conventional ages; therefore, calf value may be less, even with a positive price slide for lighter calves (Story et al., 2000). To avoid revenue shortfalls, calves can be retained and grown before selling. Feeding grain-based diets to calves less than 125 d of age has been associated with excessive fat accumulation early in the feeding period and decreased carcass weights compared with calves that enter the feedlot after 200 d of age (Schoonmaker et al., 2002). Conversely, growth of early-weaned calves can be highly efficient when compared with calves weaned at conventional ages (Peterson et al., 1987). Marked improvements in feed efficiency have been noted when grain-based finishing diets were limit-fed (Zinn, 1986; Murphy and Loerch, 1994; Schmidt et al., 2005) to early-weaned calves. Thus, high feed costs and early fat deposition may be controlled by limit-feeding a grain-based diet to early-weaned calves. Our goal was to measure performance and efficiency of light-weight, early-weaned, beef calves during an 84-d post-weaning growing period when feed intakes were varied to achieve targeted ADG of 0.45, 0.90, or 1.35 kg / day.

Materials and Methods

Animal care practices used in this study were approved by the Kansas State University Animal Care and Use Committee (protocol no. 3175).

Angus × Hereford calves (n = 243; initial BW = 156 ± 31 kg) originating from the Kansas State University commercial cow-calf herd in Hays, KS were used in this experiment. Calves were weaned at 113 ± 17 d of age. All calves were de-horned and steer calves were castrated prior to 60 d of age. At weaning, calves were weighed individually and assigned randomly to a common diet (Table 1) fed to achieve 1 of 3 rates of gain: 1) 0.45 kg ADG (**LOGAIN**), 2) 0.90 kg ADG (**MIDGAIN**), and 3) 1.35 kg ADG (**HIGAIN**). Growth and health performance were evaluated during an 84-day growing period. Targeted intakes were 1.5 % of BW for the LOGAIN diet, 2.0% of BW for MIDGAIN and 2.5% of BW for HIGAIN.

Table 1. Composition of the growing diet

Ingredient composition	% of DM
Dry-rolled sorghum grain	52.9
Dried distillers grains	23.8
Sorghum silage	18.0
Supplement*	5.3
Nutrient composition	**Amount**
CP, % of DM	15.6
NE_m, Mcal/kg	1.81
NE_g, Mcal/kg	1.20

*Supplement contained Ca, urea, ammonium sulfate, Na, Rumensin® 80, and Tylan® 40

At weaning, calves were stratified by sex and assigned to 1 of 18 pens (3 pens·sex^{-1}·treatment^{-1}; minimum area = 200 m^2/calf, bunk space = 0.46 m/calf). Animals were fed a common diet once daily at 0800. Diet formulation software predicted calves to gain ~1.35 kg/hd/d at maximal intake; we restricted the intake of the LOGAIN and MIDGAIN calves to a level that decreased their software-predicted ADG to 0.45 kg/hd/d and 0.90 kg/hd/d, respectively.

Calves were weighed individually and given initial vaccinations against respiratory pathogens (Bovi-Shield Gold® 5, Pfizer Animal Health, Exton, PA), clostridial pathogens (Ultrabac® 7, Pfizer Animal Health, Exton, PA), and *H. somnus* (Somubac®, Pfizer Animal Health, Exton, PA) at the time of maternal separation. In addition, all calves were treated for internal and external parasites (Ivomec®, Merial Limited, Atlanta, GA). Booster vaccinations were administered 14 d later. Calves were not implanted during the study.

Calf BW were measured at weaning and every 28 d thereafter for the duration of the study. Initial feed allowances were determined based on weaning BW and targeted rates of gain. Feed deliveries were adjusted every 28 d to match observed rates of gain. Carcass characteristics (12th rib fat thickness, LM depth, and marbling) were determined via ultrasound using an Aloka 500V (Aloka Co., Ltd, Wallingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-125 mm window) at the end of the 84-d growing period.

All calves were monitored for symptoms of respiratory disease twice daily during the study by trained personnel. Calves with clinical signs of BRD were removed from pens and evaluated. Calves were assigned a clinical morbidity score (scale: 1 to 4; 1 = normal, 4 = moribund), weighed, and assessed for fever. Calves with a clinical illness score > 1 and a rectal temperature > 40.0 °C were treated with therapeutic antibiotics according to label directions (1st incidence = Baytril®, Bayer Animal Health, Shawnee Mission, KS; 2nd incidence = Nuflor®, Merck Animal Health, Summit, NJ). Cattle were evaluated 72 h post-treatment and re-treated based on observed clinical signs.

Animal performance, intake, and ultrasound data were analyzed as a completely randomized design with pen as the experimental unit (PROC MIXED; SAS Inst. Inc., Cary, NC). No treatment*sex interactions were detected (P > 0.05; thus sex was removed from the final analysis. Pen within treatment was used as the random term. Incidence of undifferentiated fever was analyzed using PROC GLIMMIX (SAS Inst. Inc., Cary, NC). All models included terms for treatment and sex. No treatment*sex interactions were detected (P > 0.05; thus sex was removed from the final analysis. Pen within treatment was used as the random term. When protected by a significant F-test (P < 0.05), least squares treatment means were separated using the method of Least Significant Difference. Treatment differences were discussed when P < 0.05; tendencies were discussed when P > 0.05 and ≤ 0.10.

Results and Discussion

Calf BW increased as feed allowance increased (P < 0.01; Table 2). Feed intake was greater (P < 0.01) for the HIGAIN treatment than for the MIDGAIN treatment; moreover, feed intake of the MIDGAIN treatment was greater (P < 0.01) than for the LOGAIN treatment (Table 2). In addition, ADG increased (P < 0.01) as feed allowance increased.

Gain efficiency did not differ (P = 0.77) among treatments. Among instances where G:F improved when intake was restricted (e.g., Schmidt et al., 2005), diet NE and MP concentrations were held constant across treatments. Other research showed no difference in (Murphy and Loerch, 1994) or a reduction in G:F (Murphy et al., 1994) when intakes of high-concentrate diets were restricted. In this study, a common diet was fed at different intakes, thus a greater proportion of energy intake was used to meet maintenance requirements in cattle fed for lesser rates of gain.

The performance-based NE content of the diet used in this study was calculated using the equations of Zinn and Shen (1998). Calves fed to gain 0.45 kg/hd/d had greater (P < 0.01) apparent dietary NEm and NEg concentrations than either calves fed to gain 0.90 or 1.35 kg/hd/d. Murphy and Loerch (1994) also noted no differences in gain efficiency but a difference in performance-based diet NE concentrations of calves that were limit-fed compared with counterparts that were fed more aggressively. They attributed differences in performance-based NE concentrations to differences in diet digestibility, as intake and digestibility are inversely related (Tyrrell and Moe, 1975). Another potential explanation is that calves with greater DMI may have had increased visceral organ weights compared with limit-fed calves, a condition which has been associated with elevated maintenance energy requirements per unit of metabolic body weight (Hersom et al., 2004).

Backfat over the 12th rib was greater in the HIGAIN calves than either the LOGAIN (P < 0.01; Table 3) or the MIDGAIN (P = 0.02) calves. In contrast, there were no differences (P = 0.14) in marbling score among treatments. Longissimus muscle depth was lesser in the LOGAIN calves than either the MIDGAIN (P = 0.04) or HIGAIN (P < 0.01) calves. Early-weaned calves offered *ad libitum* access to a high-concentrate diet after weaning had poorer performance during finishing and achieved a predetermined backfat end point at lighter BW than calves weaned at conventional ages (Schoonmaker et al., 2004). These authors reported that early-weaned cattle reached

physiological maturity at a lighter-than-expected BW. Other work noted increased marbling scores in early-weaned calves limit-fed concentrates during the growing phase (Meyer et al., 2005) but calves were fed to a common-age end point in that study. Thus, the criteria used to determine harvest date may strongly influence carcass measurements.

If the harvest decision is based on ultrasonic measurement of carcass composition, early-weaned cattle may be smaller potentially and potentially produce less kg of beef per carcass than conventionally-weaned contemporaries. This may reduce beef production potential of early-weaned calves. One of the goals of this trial was to utilize restricted feeding to overcome this phenomenon, while minimizing the amount of forage fed. Meyer et al. (2005) noted increased HCW and marbling score in calves weaned 112 d before conventional weaning age and subsequently finished in a calf-fed system.

Incidence of undifferentiated fever was not different among treatments ($P = 0.95$) and was relatively mild overall (< 6%). Previous research found no differences in the health of early-weaned calves compared to calves weaned at conventional ages (Myers et al., 1999; Arthington et al., 2005; Arthington et al., 2008). Calves in the aforementioned studies were kept on pasture for a period of time after early weaning. Studies in which early-weaned calves were placed in a feedlot after weaning did not report health data (Schoonmaker et al., 2002; Schoonmaker et al., 2004). Other studies involving limit-fed, early-weaned calves also did not report health data (Murphy and Loerch, 1994; Schoonmaker et al., 2003). Based on our results, it appeared that limit-feeding early weaned calves in a feedlot did not affect subsequent health performance during an 84-d growing period.

Implications

Light-weight, early-weaned calves fed a grain-based diet at restricted rates did not exhibit improved efficiency relative to full-fed counterparts. In addition, there appeared to be limitations associated with predicting feed intake and performance of light-weight, early-weaned calves fed a grain-based diet. Our treatments influenced body composition, which may have an impact on finishing performance.

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Table 2. Growth performance of early-weaned beef calves fed a common diet to achieve 1 of 3 targeted weight gains during an 84-d growing period

	Targeted ADG			
Item,	0.45 kg/d	0.90 kg/d	1.35 kg/d	SEM
Weaning weight, kg	155	155	157	7.7
Weight at end of 84-d, kg	201[a]	213[a]	231[b]	5.8
ADG, kg/d	0.55[a]	0.69[b]	0.88[c]	0.043
DMI, kg/d	2.65[a]	3.48[b]	4.32[c]	0.001
Gain:feed	0.208	0.199	0.205	0.0138
Performance-based NE calculations[1]				
NE_m, (units; Mcal/kg)	1.93[a]	1.65[b]	1.59[b]	0.052
NE_g, (units; Mcal/kg)	1.28[a]	1.03[b]	0.98[b]	0.044

[a, b] Means within rows without common superscripts differ ($P \leq 0.05$)
[1] Calculations based on the equations of Zinn and Shen (1998)

Table 3. Carcass and health characteristics of early-weaned beef calves fed a common diet to achieve 1 of 3 targeted weight gains during an 84-d growing period

	Targeted ADG			
Item,	1 lb/d	2 lb/d	3 lb/d	SEM
Backfat over the 12th rib, mm	3.36[a]	3.55[a]	4.13[b]	0.218
Marbling, % of LM area	4.75	4.68	4.56	0.092
Muscle depth over the 12th rib, mm	38.71[a]	40.24[b]	41.45[b]	0.527
Incidence of undifferentiated fever, %	4.89	6.05	5.85	3.046

[a, b] Means within rows without common superscripts differ ($P < 0.05$)

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RELATIONSHIP OF ARRIVAL BOVINE RESPIRATORY DISEASE TITERS AND SUBSEQUENT MORBIDITY AND PERFORMANCE OF NEWLY RECEIVED CATTLE

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ABSTRACT: This study was designed to evaluate titer levels for three major precursors to Bovine Respiratory Disease (**BRD**), Infectious Bovine Rhinotracheitis (**IBR**), Bovine Virus Diarrhea (**BVD**), Parainfluenza-3 (**PI-3**), over the course of a 56 d feeding period. Five hundred and thirty-three crossbred steer calves (Initial BW = 252 ± 1.4 kg) were used in a completely randomized design. Four loads of approximately 90 head of cattle per load were obtained from New Mexico ranches with differing calf health management protocols and two loads (n = 90/load) of cattle originating from Texas livestock auctions with unknown calf health management programs. On d 0, cattle were weighed, bled and randomly assigned to one of six pens within load. Cattle were then bled and weighed on d 28 and 56. Cattle were accessed daily for symptoms of BRD and treated accordingly. At the conclusion of the study, a subset of animals not treated (**Healthy**; n = 46) were selected to serve as a control group for comparative analysis to treated cattle (**Morbid**; n = 44). Serum was analyzed for BRD antibody titers. Overall morbidity was 8.3%. Average daily gain for healthy cattle was greater ($P <$ 0.001) than morbid between d 0 and 28 and d 0 and 56. Irrespective of health status 87.8% and 28.5% ($P < 0.01$) of cattle arrived with no serum titer for IBR and PI-3, respectively. Arrival (d 0) antibody titers for BVD did not differ ($P = 0.54$) across morbid and healthy cattle. However, d 0 serum antibody titers for IBR and PI-3 differed ($P \leq 0.08$) in morbid cattle and varied across sources of cattle ($P < 0.001$), which was likely due to differences in calf management at the ranch. Day 28 serum IBR antibody titers were greater ($P = 0.05$) for morbid cattle than healthy cattle with no differences being observed for BVD or PI-3 ($P \geq 0.38$). Health and nutritional management of calves at the ranch may have an influence on arrival antibody titers to viruses involved in the BRD complex, which may increase the risk of respiratory disease in the feedlot irrespective of health programs implemented on arrival.

Key words: disease, health, management, steer

INTRODUCTION

The ability of a calf to mount a response to immunization is critical for their ability to remain healthy during the feedlot phase. Vaccination is used to stimulate an immune response, which does not necessarily imply that an animal has been completely immunized and therefore is not 100% protected against pathogens (Callan, 2001). Parker et al. (1993) compared calves that were either vaccinated or not vaccinated at branding for the Bovine Respiratory Disease complex (**BRD**) and subsequent health status at the feedlot. Approximately 65% of the calves that were vaccinated at branding had a positive titer response to Bovine Virus Diarrhea (**BVD**) by d 14 in the feedlot, whereas 33% of the calves that were not vaccinated at branding showed a positive titer response. The lack of ability to achieve 100% seroconversion in seemingly well-vaccinated calves indicates that there are underlying factors affecting responsiveness to vaccinations.

Although not well documented in peer-reviewed literature, feedlots often pay a lower price for calves from New Mexico because these calves are thought to be more susceptible to illness in the feedlot as compared to calves from other states. To date, very little literature is available where serological panels for the BRD complex have been collected from calves prior to entry into the feedlot and compared to actual incidence of morbidity and mortality, particularly in calves from New Mexico and other states. Therefore, our hypothesis is that calf serological panels' at arrival to the feedlot will identify calves that may be at high risk of becoming morbid. Our second hypothesis is that cow/calf management may have an influence on calf health performance in the first 56 d of the feeding period. Our objectives were to assess cattle serum antibody titers for the BRD complex and to compare growth performance in healthy versus morbid cattle that originated from New Mexico Ranches and South Texas Livestock Auctions.

MATERIALS AND METHODS

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

Animals

This study was conducted at the Clayton Livestock Research Center (Clayton, NM) using 533 crossbred newly-received steer calves (Initial BW = 252 ± 1.4 kg) in a completely randomized design. Four loads (84 – 90 head/load) of cattle were obtained from New Mexico ranches with differing management protocols (Figure 1) and two loads (n = 90/load) of cattle originating from Texas livestock auctions with unknown management programs.

Management and sampling

Upon arrival, cattle were placed in receiving pens overnight and had ad libitum access to water, a highly palatable starter ration, and wheat hay. Approximately, 20 h after arrival (d 0), cattle were vaccinated for infectious bovine Rhinotracheitis (IBR) /parainfluenza-3 virus (PI3)/ bovine viral diarrhea/bovine respiratory syncytial virus (Vista 5 Merck Animal Health, Summit, NJ; 2 mL SQ) and clostridial organisms (Calvary 9 Merck Animal Health, 2 mL SQ.) and treated for parasites with Fenbendazole (Safe-Guard Suspension 10%, Merck Animal Health). Steers were implanted with a Revalor IS implant (80 mg trenbolone acetate and 16 mg estradiol, Merck Animal Health).

Management program	NM Ranch 1	NM Ranch 2	NM Ranch 3	NM Ranch 4	TX Auction 5	TX Auction 6
Vaccinate cows					Unknown	Unknown
Mineral fed to cows					Unknown	Unknown
Vaccinate calves at branding					Unknown	Unknown
Vaccinate calves at weaning					Unknown	Unknown
Precondition calves					Unknown	Unknown

Figure 1. Ranch management practices.

Individual BW and rectal temperature were taken during processing and an individual ear identification tag was given to each steer. Additionally, 9 mL of blood was collected from the jugular vein into a Vacuette tube with serum clot activator (Greiner Bio-One, Kremsmuenster, Austria). Calves were dehorned and castrated as needed. Cattle were randomly assigned to one of two metaphylactic treatments: 1) 5.5 mL of tulathromycin (Draxxin, Zoetis Animal Health, Madison, NJ); 2) 5.0 mL tildipirosin (Zuprevo, Merck Animal Health). Calves were then assigned to one of 6 pens within load (approximately 15 head/pen, 3 pens/treatment). Cattle were again bled and individually weighed and rectal temperatures were recorded on d 28 and 56. Cattle were fed twice daily at 0700 h and 1300 h one of 2 diets over the course of the study, 1) starter ration from d 0-28 (19.4% CP, 1.9 Mcal/kg NEm and 1.30 Mcal/kg NEg); 2) grower ration from d 29 - 56 (18.1 % CP, 1.94 Mcal/kg NEm and 1.32 Mcal/kg NEg). Upon treatment with metaphylaxis cattle were placed on a 5 day moratorium before they were eligible for second treatment. Cattle were observed throughout the daylight hours for symptoms of BRD and treated accordingly. Cattle were pulled from their pen based on observed signs of sickness (e.g. lethargy, nasal discharge or abnormal activity). Cattle pulled were moved to processing facility where BW and rectal temperature were measured. Cattle that had lost BW since last weighing or had a temperature of ≥40.5 °C or any other abnormal respiration pattern were treated. When required, steers on the Draxxin treatment were medicated with ceftiofur crystalline (3.3mL/100 kg BW, Excede, Pfizer Animal Health) and those on Zuprevo treatment received florfenicol and fluninixin meglumine (13.2 mL/100 kg BW, Resflor Gold, Merck Animal Health). All animals were returned to pen after treatment. If after 72 hours a second treatment was required, cattle were administered enroflaxacin (12.5 mL/100 kg BW, Baytril, Bayer Animal Health, Shawnee Mission, KS). At the conclusion of the study a subset of animals not treated (**healthy**) were selected to serve as a control group for comparative analysis to treated cattle (**morbid**).

Analysis

After collection, blood was placed in a refrigerator at 4 °C for 3 h, and serum was separated by centrifugation at 1,000 x *g* for 20 min. Serum was then decanted and stored at -10 °C for subsequent analysis. Upon completion of the study, serum samples for healthy cattle (n = 46) and morbid cattle (n = 44) were packaged and shipped overnight to the Oklahoma Animal Disease Diagnostic Center (Stillwater, OK) for assay of serum IBR, BVD, and PI-3 antibody titers.

Statistical analysis

Average Daily Gain data was analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Serological data was analyzed using PROC GLIMIX (SAS Inst. Inc.). Model included the influence of metaphylaxis, origin of cattle and all possible interactions. There was no metaphylaxis or metaphylaxis × health status observed ($P \geq 0.16$). Therefore metaphylaxis was removed from model and only the effects of health status will be reported.

RESULTS AND DISCUSSION

Performance and Health

Performance data during the 56 d feeding period is shown in Table 1. No difference in BW between healthy and morbid cattle was observed for d 0 ($P = 0.98$). However healthy cattle were larger ($P = 0.09$) at d 28 as well as d 56. Average daily gain was greater ($P < 0.001$) for healthy cattle during d 0 – 28 compared to morbid cattle. However, no differences in ADG were observed ($P = 0.79$) between healthy and morbid cattle during d 29 – 56. Nevertheless, overall performance (d 0 – 56) was greater ($P < 0.001$) in healthy cattle due to the differences in ADG during the first 28 d. Specifically, the improved performance in morbid cattle during the second 28 d period could not compensate for the loss during the first 28 d. This is in agreement with Fulton et al. (2002) who reported that cattle who were morbid had lower ($P = 0.008$) ADG.

The number of cattle and health status are described in Table 2. A total of 44 head or 8.3% were treated over the course of the 56 day trial. Number of cattle treated per source of cattle ranged from 5 to 14 head, percentages ranged from 5.5% to 16.9%. Differences in treatment rates may have resulted from differences in pre-arrival vaccination and nutritional management programs. In a similar study Martin and Bohac (1986) showed treatment rates of 5 different sources of cattle that ranged from 13.8% to 64.4%.

Serological

Differences in mean IBR, BVD and PI-3 serum antibody titer levels between healthy and morbid beef cattle across 56 d feed period are shown in Table 3. Mean serum antibody titer levels for BVD did not differ ($P \geq 0.34$)

between healthy and morbid cattle across the 56 d study. These results agree with Martin and Bohac (1986) who showed showed no difference in arrival serum antibody titers for BVD between healthy and morbid cattle. In the current experiment, arrival PI-3 antibody titers were greater ($P = 0.07$) for healthy cattle as compared to morbid cattle. However, there was no difference ($P \geq 0.32$) in antibody titers between healthy and morbid cattle for d 28 and 56. This is in agreement with morbidity data, with all but two steers requiring treatment only during the first 28 d.

Health management of calves at the ranch may have an influence on arrival antibody titers to viruses involved in the BRD complex, which may increase the risk of respiratory disease in the feedlot irrespective of health programs implemented on arrival. Parker et al. (1993) showed no difference ($P > 0.29$) in feedlot arrival serum antibody titer levels between calves that were either vaccinated or were not vaccinated for viruses of the BRD complex at branding and no vaccinations for either group were administered at weaning. Yet, by day 28, 73% of the calves that were vaccinated at branding had a positive serum antibody titer for BVD compared 42% for calves that were not vaccinated at branding. Thereby indicating that calves vaccinated at branding responded to arrival vaccinations at greater level than those not vaccinated at branding.

Differences in arrival IBR, BVD and PI-3 serum antibody titer levels across ranches are show in Table 4. Irrespective of health status cattle originating from ranch 2 had higher ($P < 0.05$) average serum antibody titer levels for IBR as compared to other ranches. Similarly ranches 1 and 2 had higher ($P \leq 0.01$) average serum antibody titer levels for BVD. Calves from ranches 1 and 2 were weaned and immediately shipped to the feedlot, whereas ranches 3 and 4 held calves at the ranch for a minimum of 45 d. Boyles et al. (2007) conducted a study to evaluate three different weaning strategies and impact on feedlot morbidity. Cattle weaned on the truck and in drylot pens had greater morbidity compared to cattle weaned on pasture. Mathis et al. (2008) reported that feedlot morbidity and mortality of cattle backgrounded on pasture versus cattle backgrounded in drylot pens. No difference was observed in morbidity between treatments, however cattle backgrounded in the pasture treatment had lower mortality compared to drylot treatment.

Furthermore, differences existed between ranch 1 and 2 in regards to vaccination program. Ranch 1 only vaccinated at branding whereas ranch 2 vaccinated at branding and boostered 3 weeks later but did not administer any vaccines at weaning. Ranches 3 and 4 vaccinated at branding and at weaning with a booster approximately 14 d later. Therefore, it is hard to ascribe a specific management program that caused differences in arrival antibody titers.

Based on our results, health and nutritional management of calves at the ranch may have an influence on arrival antibody titers to viruses involved in the BRD complex. However, due to low number of cattle and relatively low incidence of morbidity in this experiment, it is difficult to identify one factor that may influence calf health in the feedlot. Future work is needed to further elucidate the management factors on the ranch that may predispose cattle to BRD in the feedlot.

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Table 1. Performance of healthy versus morbid cattle

	Status			
	Healthy	Morbid	SE	*P*-value
BW, kg				
Day 0	236.9	236.8	4.4	0.98
Day 28	263.7	251.6	4.8	0.08
Day 56	305.2	292.6	5.3	0.09
ADG, kg				
Day 0-28	0.97	0.54	0.104	<0.001
Day 29-56	1.46	1.44	0.102	0.79
Day 0-56	1.21	0.99	0.084	<0.001

Table 2. Number of cattle by ranch and subsequent health status

	Ranch[1]						
	1	2	3	4	5	6	Total
No. at arrival	90	90	83	90	90	90	533
Treated, Hd							
d 0-28	5	6	14	5	5	8	43
d 29-56	0	0	0	1	0	0	1
Treated Twice, Hd							
d 0-28	0	0	1	0	0	0	1
d 29-56	0	0	0	0	1	0	1
Total treated, % [2]	5.5%	6.7%	16.9%	6.7%	5.5%	8.9%	8.3%

[1] Ranch corresponds to the ranch in which the cattle were sourced. Ranches 1 through 4 were sourced from New Mexico ranches. Ranches 5 and 6 were sourced from south Texas livestock auctions.
[2]Total treated: the sum of treated d 0-28 and d 29-56 divided by total number of head at arrival.

Table 3. Differences in IBR, BVD and PI-3 serum antibody titer levels between healthy and morbid beef cattle across 56-d feeding period

	Status			
Item	Healthy	Morbid	SE	*P*-Value
IBR[1]				
d 0	0.46	2.13	0.67	0.08
d 28	7.69	16.5	3.2	0.05
d 56	5.2	12.1	2.6	0.06
BVD[2]				
d 0	54.5	63.1	10.2	0.54
d 28	182.1	200.9	15.4	0.38
d 56	182.7	201.2	14.0	0.34
PI-3[3]				
d 0	85.1	51.8	12.9	0.07
d 28	151.4	140.6	13.8	0.57
d 56	151.5	131.3	14.6	0.32

[1] *Infectious Bovine Rhinotracheitis.*
[2] *Bovine Viral Diarrhea.*
[3] *Parainfluenza-3.*

Table 4. Differences in IBR, BVD and PI-3 serum antibody titer levels across load

	Ranch[1]						
	1	2	3	4	5	6	SE
IBR[2]	0^{a}	$5.17^{b,c}$	0.82^{a}	$1.58^{a,d}$	0^{a}	0.19^{a}	1.27
BVD[3]	163.3^{a}	129.0^{a}	10.3^{b}	15.6^{b}	32.0^{b}	2.7^{b}	19.2
PI-3[4]	53.9^{a}	56.3^{a}	60.8^{a}	186.4^{b}	29.1^{a}	24.2^{a}	24.4

[1] Load corresponds to the ranch in which the cattle were sourced. Ranches 1 through 4 were sourced from New Mexico ranches. Ranches 5 and 6 were sourced from south Texas livestock auctions.
[2] *Infectious Bovine Rhinotracheitis.*
[3] *Bovine Viral Diarrhea.*
[4] *Parainfluenza-3.*
[ab] Means within row differ at $P \leq 0.01$.
[cd] Means within row differ at $P \leq 0.05$.

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COVER SYSTEMS FOR WET DISTILLER'S GRAIN STORED IN CONCRETE BUNKERS

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ABSTRACT: The objective of this project was to evaluate the effects of different cover systems for wet distiller's grain (WDG) stored in concrete bunkers on nutrient quality and product loss during an extended storage period. Six concrete bunkers, designed to hold, approximately 22.7 metric tons of WDG were assigned randomly to one of three cover system treatments 1) No cover (Uncovered); 2) 6 mil black plastic and tires (Plastic) 3) 0.91kg stock salt/929 cm2 of WDG surface area (Salt). Cover system treatments were applied to WDG immediately following delivery. Samples of WDG were obtained on d 0 (the day of arrival) and every 28 d thereafter, for the duration of the study (196 d), from 2 randomly selected locations within each bunker using a commercially available grain probe. At the end of the storage period WDG was removed from bunkers and weighed to determine total product loss. All samples were submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, and ADF content. A day × cover system interaction ($P \leq 0.05$) was observed for DM and CP content of WDG. Dry matter content of WDG covered with Plastic or Salt was not different at any time point during the 196 d storage period ($P > 0.05$). However, DM content of Uncovered WDG was more variable and ranged from 34.6 to 50.4%. Crude protein content was also not different ($P \geq 0.38$) between Plastic and Salt covered WDG at any time point; but increased over time in Uncovered WDG (day × cover; $P < 0.05$). Duration of storage also affected ADF content of stored WDG (day; $P < 0.01$). Total product loss (DM basis) was greatest for Uncovered, intermediate for Salt and the least for Plastic covered WDG (73.3, 35.2 and 20.5 % of initial, respectively; cover $P < 0.01$) during the 196 d storage period. These data indicate that, under the conditions of our study, Uncovered WDG will deteriorate over 196 d. However, Plastic and Salt are both effective cover systems for WDG stored in concrete bunkers.

Key Words: Distillers grain, bunker, storage

Introduction

The use of wet distiller's grains **(WDG)** as a feedstuff in livestock operations has increased substantially, but has been limited primarily to livestock producers with the capability of utilizing truckload quantities (approximately, 22.7 metric tons) of WDG within 7 to 21 days due to the short shelf life of WDG. The high moisture content of WDG (35% DM) has restricted the use of conventional methods of storing high moisture feedstuffs, as WDG alone cannot be mechanically compacted to exclude oxygen. Therefore, forages or other feedstuffs are often utilized as bulking agents to facilitate mechanical packing of WDG (Kalsheur et al., 2002; Adams et al., 2008). However, Waggoner et al., (2011) demonstrated that WDG could be stored in concrete bunkers for up to 208 d, without the addition of bulking agents or packing, when covered with 6 mil black plastic and tires. Covering large piles of WDG with plastic can be tenuous and producers are interested in other low cost covering methods or leaving piles uncovered.

Therefore, the objective of this study was to evaluate the effects of different cover systems (1. No cover; 2. Plastic; 3. Stock salt) for WDG stored in concrete bunkers on nutrient quality and product loss (shrink) during an extended storage period.

Materials and Methods

Six identical concrete bunkers (4.1 m (width) × 12.0 m (length) designed to hold approximately 22.7 metric tons (1 truckload of WDG) were assigned randomly to one of three cover system treatments: 1) No cover (**Uncovered**); 2) 6 mil black plastic and tires (**Plastic**) 3) 0.91kg stock salt/929 cm^2 of WDG surface area (**Salt**). Loads of WDG were delivered over 2 days (3 loads/d, 1 of each cover system treatment/d). Covering system treatments were applied within 1 h of delivery on the day of arrival.

Samples were obtained from each load of WDG on the day of arrival and every 28 d thereafter, for the duration of the study (196 d), from 2 randomly selected locations within each bunker using a commercially available grain probe. Samples were obtained at a common depth of approximately 50 cm, were composited within bunker and frozen for later analysis. At the conclusion of the project samples were submitted to a commercial laboratory (SDK Labs, Hutchinson, KS) for analysis of DM, CP, and ADF content.

At the conclusion of the storage period, all remaining WDG was individually removed from each bunker and weighed for determination of total product loss (shrink). Total product loss was determined by difference from the initial delivery load weight.

Statistical Analysis. All data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The effects of cover, day, and cover × day on DM, CP, ADF, and product loss were evaluated as repeated measures design (covariance structure = autoregressive order one). Bunker served as the replicate. Data are presented as least squares means and differences were considered significant at $P < 0.05$.

Results and Discussion

Physical Observations. After 196 d in storage the Uncovered WDG had substantially eroded. Large, deep cracks penetrated approximately two thirds of the depth of the pile. A thin layer of mold had developed over the surface of Plastic covered WDG. Upon further examination the WDG beneath the mold layer was relatively well preserved. A few small surface cracks were apparent on the surface of the Salt covered WDG, but did not appear to have penetrated more than a few cm below the surface. The Salt had dissolved into the WDG effectively sealing the WDG.

Nutrient Analysis. A day × cover interaction ($P \leq 0.05$) was observed for both DM and CP content of WDG (**Table 1 and 2**). Dry matter content of WDG covered with Plastic or Salt were not different at any time point during the 196 day study ($P > 0.05$). However, DM content of WDG that was left Uncovered was more variable and ranged from 34.2 to 50.4% during the course of the study. Crude protein content was also not different ($P \geq 0.38$) between Plastic and Salt covered WDG at any time point; but increased over time in Uncovered WDG from 38.2 to 52.5 % CP (day × cover; $P < 0.05$).

An effect of day ($P < 0.01$) was observed for ADF of stored WDG (Table 3). The maximum observed change in ADF among cover system treatments was 3.2 ADF units, which likely indicates that the relative composition of hemi-cellulose, cellulose and lignin within stored WDG may be changing

Shrink. Total product loss (DM basis) was greatest for Uncovered, intermediate for Salt and the least for Plastic covered WDG (76.3, 35.2 and 20.5 % of initial, respectively; cover $P < 0.01$) during the 196 d storage period. Christensen et al. (2010) reported DM losses of 15.3% for uncovered, 0% for plastic and 3.6% salt covered mixtures of WDG (70%) and cornstalks (30%) packed into barrels and stored for 57 d. These results are considerably lower than those observed in the present study. However, the large differences in DM shrink reported may be attributed to the substantial difference in scale as the present study was conducted at field scale (8,000 kg/bunker vs. 52 kg/barrel). However, the relative ranking of the different cover systems was similar.

The observed variation in DM and CP combined with amount of produce loss (76.3%) of Uncovered WDG clearly demonstrates that WDG will deteriorate if exposed to the elements for extended periods of time. Dixon and Combellas (1983) also evaluated the use of salt as a cover system for wet brewer's grain in Venezuela and concluded, salt was not an effective cover system in a humid tropical climate. Although, Salt covered WDG did experience approximately 15% more product loss compared to Plastic. The lack of difference in DM and CP content of Plastic and Salt covered WDG over 196 d suggests that Salt and Plastic may both be effective cover systems for WDG in semi-arid climates.

Table 1: Effect of storage and cover system on DM content of wet distillers grain stored in concrete bunkers (day × cover; $P \leq 0.05$).

	Treatment[1]			
Day	Uncovered	Plastic	Salt	SEM
0	38.2[a]	36.1[a]	38.0[a]	1.10
28	40.0[a]	38.1[b]	38.1[b]	
56	42.4[a]	35.4[b]	37.5[b]	
84	43.6[a]	35.5[b]	36.4[b]	
112	48.0[a]	36.3[b]	37.3[b]	
140	50.4[a]	35.7[b]	37.2[b]	
168	35.3[a]	35.6[a]	37.5[a]	
196	34.2[a]	34.4[a]	35.4[a]	

[1]Treatments: 1) No cover (**Uncovered**); 2) 6 mil black plastic and tires (**Plastic**); 3) 0.91kg stock salt/929 cm^2 of WDG surface area (**Salt**).
[a,b] Means without a common letter differ $P \leq 0.05$.

Table 2: Effect of storage and cover system on CP content of wet distillers grain stored in concrete bunkers (day × cover; $P \leq 0.05$).

	Treatment[1]			
Day	Uncovered	Plastic	Salt	SEM
0	35.8[a]	33.1[a]	33.4[a]	1.56
28	36.1[a]	34.1[a]	35.4[a]	
56	37.8[a]	33.9[b]	37.8[a]	
84	43.6[a]	33.9[b]	35.3[b]	
112	41.2[a]	34.5[b]	36.5[b]	
140	50.5[a]	34.5[b]	35.1[b]	
168	51.0[a]	35.0[b]	34.8[b]	
196	52.5[a]	36.2[b]	35.9[b]	

[1]Treatments = 1) No cover (**Uncovered**) 2) 6 mil black plastic and tires (**Plastic**) 3) 0.91kg stock salt/929 cm^2 of WDG surface area (**Salt**).
[a,b] Means without a common letter differ $P \leq 0.05$.

Table 3: Effect of storage and cover system on ADF content of wet distillers grain stored in concrete bunkers (day $P < 0.01$; cover $P = 0.10$; day × cover; $P = 0.25$).

	Treatment[1]			
Day	Uncovered	Plastic	Salt	SEM
0	20.0	19.3	18.0	1.35
28	19.6	19.4	19.4	
56	19.4	19.5	18.5	
84	23.1	19.1	19.9	
112	20.6	19.6	20.6	
140	27.8	20.9	20.0	
168	24.0	20.5	21.1	
196	23.0	22.5	20.2	

[1]Treatments = 1) No cover (**Uncovered**) 2) 6 mil black plastic and tires (**Plastic**) 3) 0.91kg stock salt/929 cm^2 of WDG surface area (**Salt**).

Table 4: Effect of storage and cover system on total product loss (Shrink) of wet distillers grain stored in concrete bunkers (DM basis; cover $P < 0.01$).

	Treatment[1]		
Item	Uncovered	Plastic	Salt
Initial Wt., kg	8,269	8,387	8,750
Final Wt., kg	1,976	6,766	5,671
Shrink Wt., kg	6,293	1,743	3,079
Shrink, %	73.3	20.5	35.2

[1]Treatments = 1) No cover (**Uncovered**) 2) 6 mil black plastic and tires (**Plastic**) 3) 0.91kg stock salt/929 cm^2 of WDG surface area (**Salt**).

Implications

These data indicate that, under the conditions of our study, Uncovered WDG will deteriorate over 196 d. However, Plastic and Salt are both effective cover systems for WDG stored in concrete bunkers.

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EFFECTS OF ZUPREVO AS MASS MEDICATION OF RECEIVING CATTLE ON FEEDLOT PERFORMANCE AND HEALTH

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ABSTRACT: Five hundred and thirty-three steer calves (225 ± 1.4 kg initial BW) were used in a randomized complete block design. The objective was to evaluate the effect of mass medication with Zuprevo at receiving on performance, morbidity, and cost of bovine respiratory disease complex (BRD) related treatment of newly-received steer calves. Treatments consisted of assigning alternate steers to 1 of 2 treatments as they passed through the processing chute: 1) calves treated with tulathromycin (2.5 mL/100 kg BW, **Draxxin**, Pfizer Animal Health); and 2) calves treated with tildipirosin (2.2 mL/100 kg BW, **Zuprevo**, Merck Animal Health). Calves were fed twice daily at 0700 and 1300, and were weighed on d 0, 28, and 56. Two different diets were offered during this experiment: 1) first diet was offered from d 0 to 28 (17.4% cracked-corn grain) 2) the second diet was offered from d 29 to 56 (33.7% cracked-corn grain). Dry matter intake tended to be greater ($P = 0.07$) for the Draxxin than Zuprevo treatment from d 0 to 56 (6.59 and 6.48 ± 0.041 kg, respectively). In addition, ADG was greater ($P < 0.03$) for Draxxin than Zuprevo from d 0 to 56 (1.41 and 1.35 ± 0.019 kg, respectively). Conversely G:F was not affected ($P = 0.12$) by mass medication treatment (0.22 and 0.21 ± 0.006). Morbidity (7.6 and 9.36 ± 1.33%, respectively) and retreatments (5.6 and 1.4 ± 4.05%, respectively) did not differ between treatments ($P \geq 0.37$). Cost of mass medication ($18.31 and $18.46 ± 0.42, respectively) and of medication of retreated animals ($0.12 and $0.11 ± 0.11, respectively) were similar ($P \geq 0.47$) between treatments. Cost of medication of morbid animals ($0.92 and $1.68 ± 0.203, respectively; $P = 0.01$) and total cost of medication ($19.36 and $20.22 ± 0.279, respectively; $P = 0.04$) were greater for Zuprevo than for Draxxin. Although DMI tended to decrease, and ADG decreased for Zuprevo, G:F was not affected by treatment during the 56-d feeding period. Moreover, there were no treatment effects on animal health responses and mass medication costs. But, the cost of medication of morbid animals and total cost of medication were greater for Zuprevo.

Key words: bovine respiratory disease, mass medication, receiving cattle

Introduction

Bovine respiratory disease (BRD) is the most economically important disease in newly weaned/received beef cattle, and is the leading cause of morbidity and mortality in feedlots (Woolums et al., 2005). Moreover, the associated losses in performance and carcass merit have an important negative economic impact on the beef cattle industry (Gardner et al., 1999). Preventive use of antibiotic programs at arriving decreases the incidence of BRD and the negative effects with which it is associated.

Including chlortetracycline in feed of newly received cattle decreased morbidity, mortality, and improved performance. But, feed intake must be adequate to provide the proper dose of antibiotic. Preventive treatment (mass medication) with antibiotics has been successful in decreasing the incidence of BRD. The combination of long-acting oxytetracycline (Terramycin 50) for 3 consecutive days, and sustained release sulfadimethoxine (Albon SR) at cattle arrival decreased morbidity (Lofgreen, 1983). Also, mass medication with florfenicol (Nuflor; Frank et al., 2002) or tilmicosin phosphate (Micotil; Galyean et al., 1995) is effective for decreasing morbidity of newly received, stressed cattle. More recently, tulathromycin (Draxxin) was more effective in decreasing the incidence of BRD when used as mass medication at feedlot receiving (Rooney et al., 2005). A dose of Draxxin provides therapeutic levels of tulathromycin for 14 d. The new antibiotic tildipirosin (Zuprevo) is available for prevention and treatment of BRD. A dose of Zuprevo provides therapeutic levels of tildipirosin for 21 d. Because of the longer action of Zuprevo, it is expected to provide longer and better protection against BRD, and promote better growth performance than Draxxin. However, limited information is available on the efficiency of Zuprevo on preventing BRD of feedlot newly received cattle. Therefore, a study was conducted to evaluate effects of mass medicating with Zuprevo on performance and health of newly received feedlot calves.

Materials and Methods

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

Five hundred and thirty three crossbreed newly-received steer calves (225 ± 1.4 kg initial BW) were used in a randomized complete block. Calves were transported from 4 different locations in New Mexico and 2 locations in

south Texas to the Clayton Livestock Research Center in Clayton, NM. Upon arrival calves had immediate access to wheat hay and water. Processing took place the morning after the day of arrival, which included vaccination (Calvary 9, and Vista 5, Merck Animal Health); treatment for parasites (Moxidextin, and Safeguard, Merck Animal Health); and ear implant with Revalor IS (80 mg trenbolone acetate and 16 mg estradiol, Merck Animal Health). Individual BW, and rectal temperature were recorded during processing, and an individual ear identification tag was assigned to each calf. Calves were dehorned and castrated as needed. As calves passed through the processing chute they were alternately assigned to 1 of 2 treatments: 1) calves treated with 2.5mg/kg of tulathromycin (2.5mL/100 kg BW, **Draxxin**, Pfizer Animal Health, New York, NY); 2) calves treated with 4 mg/kg of tildipirosin (2.2 mL/ 100 kg BW, **Zuprevo**, Merck Animal Health, DeSoto, KS). Calves within treatments were then assigned to pens (15 head/pen; 18 pens/treatment). Animals were blocked according to arriving date, which corresponds to the location from which they came. Therefore, there were 6 arrival date blocks.

Two diets were offered to steers during the study. One diet was offered from d 0 to 28, and a second diet was offered from d 29 to 56. The first diet was an 85% concentrate diet with 43.3% corn gluten feed, 18.2% corn distiller grains with solubles, and 17.4% cracked corn. The second diet was an 88% concentrate diet with 34.1% corn gluten feed, 14.1% corn distiller grains with solubles, and 33.7% cracked corn. Calves were fed twice daily at 0700 and 1300, and were monitored daily for symptoms of BRD, including decreased feed intake, cough, nasal discharge, increased respiratory rate, and depression. Animals observed with these symptoms were removed from their pen and further examined. If rectal temperature was $\geq$ 40.5 °C, the animal was medicated. When required, steers on the Draxxin treatment were medicated with 6.6 mg/kg of ceftiofur crystalline (3.3 mL/100 kg BW, Excede, Pfizer), and those on Zuprevo treatment received 39.6 mg/kg of florfenicol and 2.178 mg/kg flunixin meglumine (13.2 mL/100 kg BW, Resflor Gold, Merck Animal Health). Animals were returned to their assigned pen following antibiotic treatment. If symptoms continued after 72 h, a second treatment was used and consisted of 12.5 mg/kg enroflaxacin (12.5 mL/100 kg BW, Baytril, Bayer Animal Health, Shawnee Mission, KS).

Body weight (unshrunk BW data measured on a single day were obtained, and BW was reduced 4% to adjust for digestive tract fill) and intake measurements were obtained on d 0, 28, and 56. Morbidity data were expressed as percentage per pen treated once, and retreated was expressed as percentage of treated once, treated twice, to be analyzed as continuous data. In calculations of medication cost a value of $3.22/mL for Draxxin and $3.54/mL for Zuprevo were included for initial mass medication of all animals. In addition, a value of $1.56/mL for Excede and $0.59/mL for Resflor Gold were used for calculation of medication cost of morbid animals. Also, a value of $0.68/mL for Baytril was included for calculation of medication cost of retreated animals. Medication cost of morbid and retreated animals was calculated as cost per pen divided by total number of animals per pen. Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Pen was the experimental unit. The model included treatment and treatment by block interaction. The random statement included block.

Results and Discussion

Effects of mass medication with Zuprevo on performance of steer calves during the receiving period are presented on Table 1. Body weight at d 0, 28, and 56 were not affected ($P \geq 0.15$) by treatment. Average daily gain from d 0 to 28 ($P = 0.28$), and d 29 to 56 ($P = 29$) were not affected by treatment. However, ADG from day 0 to 56 decreased ($P = 0.03$) for Zuprevo. Similar to ADG, DMI was not affected from d 0 to 28 ($P = 0.20$), and d 29 to 56 ($P = 17$), and from 0 to 56 tended to decrease ($P = 0.07$) for Zuprevo. Even though the means for ADG and DMI were lower for Zuprevo, differences were small and we consider them of little biological significance. The G:F ratio was not affected ($P \geq 0.12$) by treatment at either d 0 to 28, d 29 to 56, or d 0 to 56. The lack of treatment effect on G:F ratio suggests similar performance of newly received cattle treated with Zuprevo or Draxxin. Previous studies reported improved performance by mass medicating with chlortetracycline (Duff et al., 2000), tilmicosin (Galyean et al., 1995) and tulathromycin (Rooney et al., 2005).

Effects of mass medication with Zuprevo on morbidity and medication cost of feedlot newly received steer calves are presented in Table 2. Morbidity ($P = 0.37$), cattle retreated ($P = 0.47$), rectal temperature on d 0 ($P = 0.90$), d 28 ($P = 0.74$), and d 56 ($P = 0.86$), cost of mass medication ($P = 0.47$), and medication cost of retreated animals ($P = 0.92$) were not affected by Zuprevo. However, medication cost of morbid animals ($P = 0.01$), and total cost of medication ($P = 0.04$) were greater for Zuprevo. Morbid calves (first time pulled) from the Draxxin treatment were medicated with Excede (3.3 mL/100 kg BW with a cost of $1.56/mL), while those from Zuprevo were medicated with Resflor Gold (13.2 mL/100 kg BW with a cost of $0.59/ml). Therefore the dose per 100 kg BW was calculated to be $5.15 for Excede, and $7.79 for Resflor Gold. Even though, morbidity was not affected by treatment, cost of medication of morbid animals was greater for Zuprevo because of the greater calculated cost of medicating with Resflor Gold than with Excede. Also, the total cost of medicating Zuprevo calves was greater than that of Draxxin because the greater calculated cost of medicating morbid animals with Resflor Gold than with Excede.

Implications

Mass medicating newly received cattle with Zuprevo is as effective as Draxxin on reducing the incidence of BRD. Although ADG and DMI decreased for Zuprevo, differences appear to have little practical significance and similar performance should be expected when mass medicating newly received cattle with Zuprevo or Draxxin. Therefore, Zuprevo is a viable alternative for mass medicating feedlot newly received cattle.

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Table 1. Effects of Zuprevo mass medication on performance of steer calves during the receiving period

	Treatments[1]			
Item	Draxxin	Zuprevo	SE	*P*-value
BW, kg				
d 0	224	226	1.4	0.15
d 28	262	263	1.6	0.51
d 56	303	302	1.7	0.84
ADG, kg				
d 0 to 28	1.37	1.32	0.033	0.28
d 29 to 56	1.46	1.39	0.047	0.29
d 0 to 56	1.41	1.35	0.019	0.03
DMI, kg				
d 0 to 28	5.42	5.34	0.040	0.20
d 29 to 56	7.81	7.67	0.070	0.17
d 0 to 56	6.59	6.48	0.041	0.07
G:F, kg/kg				
d 0 to 28	0.25	0.25	0.006	0.46
d 29 to 56	0.19	0.18	0.006	0.48
d 0 to 56	0.22	0.21	0.003	0.12

[1]Mass medicated steers received a subcutaneous injection in the neck at receiving or d 0 of the experiment: Draxxin = steers received tulathromycin 2.5 mg/kg (2.5 mL/100 kg BW, Draxxin, Pfizer Animal Health, New York, NY); Zuprevo= steers received tildipirosin 4 mg/kg (2.2 mL/100 kg BW, Zuprevo, Merck Animal Health, DeSoto KS).

Table 2. Effects of Zuprevo mass medication on morbidity and medication cost during the receiving period

	Treatments[1]			
Item	Draxxin	Zuprevo	SE	*P*-value
Morbidity, %[2]	7.6	9.3	1.33	0.37
Retreatment, %[3]	5.6	1.4	4.05	0.47
Rectal temperature, °C				
d 0	39.5	39.5	0.032	0.90
d 28	39.4	39.4	0.060	0.74
d 56	38.8	38.7	0.064	0.86
Cost of mass medication, $[4]	18.31	18.46	0.42	0.47
Medication cost of morbid animals, $[5]	0.92	1.68	0.203	0.01
Medication cost of retreated animals, $[6]	0.12	0.11	0.114	0.92
Total cost of medication, $[7]	19.36	20.22	0.279	0.04

[1]Mass medicated steers received a subcutaneous injection in the neck at receiving or d 0 of the experiment: Draxxin = steers received tulathromycin 2.5 mg/kg (2.5 mL/100 kg BW, Draxxin, Pfizer Animal Health, New York, NY); Zuprevo = steers received tildipirosin 4 mg/kg (2.2 mL/100 kg BW, Zuprevo, Merck Animal Health, DeSoto, KS).

[2]An animal was considered morbid if it was treated for bovine respiratory disease (BRD). Morbidity was calculated as % of animals treated once per pen.

[3]Retreatment was calculated as number of cattle treated 2 times for bovine respiratory disease/total number of cattle treated once.

[4]Mass medication cost calculation included a value of $3.22/mL for Draxxin and $3.54/mL for Zuprevo.

[5]Calculation of medication cost of morbid animals included a value of $1.56/mL for Excede (Pfizer Animal Health) and $0.59/mL for Resflor Gold (Merck Animal Health). Morbid animals from the Draxxin treatment were medicated with Excede and morbid animals from the Zuprevo treatments were medicated with Resflor Gold. Cost of morbid animals treated per pen was divided by number of animals in the pen.

[6]Calculation of medication cost of retreated animal included a value of $0.68/mL for Baytril. Cost of retreated animals treated per pen was divided by number of animals in the pen. Animals from both treatments were medicated with Baytril (Bayer Animal Health, Shawnee Mission, KS) when retreatment was required.

[7]Total cost of medication was calculated by adding cost of mass medication, medication cost of morbid animals, and medication cost of retreated animals.

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IMPACT OF SUPPLEMENTAL PROTEIN SOURCE ON ADG, FEED INTAKE, CALF BIRTH BW, AND REBREEDING IN PREGNANT BEEF HEIFERS

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ABSTRACT: A 3-yr study was conducted to determine the effect of supplemental protein source on ADG, feed intake, calf birth BW, and subsequent pregnancy rate in pregnant beef heifers. Crossbred, Angus-based, AI-pregnant heifers (yr 1 n = 38, yr 2 n = 40, yr 3 n = 36) were stratified by BW (450 ± 10 kg) and placed in a Calan Broadbent individual feeding system at approximately d 142 of gestation. Following a 25 d adaptation period, an 84 d feeding trial was conducted. Heifers were offered ad libitum grass hay (8 to 11% CP, DM basis) and no supplement (CON), 0.83 kg/d distillers based supplement (HI), or 0.83 kg/d dried corn gluten based supplement (LO). Supplements were formulated to be isocaloric, isonitrogenous (28% CP, DM basis), and equal in lipid content; but differed in rumen undegradable protein. Dry matter intake was also calculated based on NE values of the feed to account for different energy levels of the supplement compared with the control diet. Heifers receiving no supplement tended (P = 0.09) to consume less total DM than either supplement treatment. Forage-only DMI was greater (P < 0.01) for CON heifers (9.94 ± 0.12 kg) compared with HI or LO heifers (8.50 and 8.34 ± 0.12 kg, respectively). Net energy DMI was less (P < 0.01) for CON heifers (4.98 ± 0.23 kg) compared with HI or LO heifers (5.43 and 5.35 ± 0.23 kg, respectively). Heifers receiving no supplement had less ADG (0.59 ± 0.14 kg) than either HI (0.82 ± 0.14 kg) or LO heifers (0.78 ± 0.14 kg, P < 0.01), resulting in lower (501 ± 9 kg) BW (P < 0.01) than HI (519 ± 9 kg) heifers at the end of the trial. Calf birth BW did not differ (P = 0.99) among treatments. At prebreeding, CON heifers weighed less (P < 0.03) than LO heifers. Cow BW was similar (P = 0.48) among treatments at pregnancy diagnosis, and final pregnancy rate was also similar (87 ± 3%, P = 0.22). Protein supplementation increased ADG in pregnant heifers; however, calf birth BW and subsequent pregnancy rates were similar.

Key words: feed intake, pregnant beef heifer, supplementation

Introduction

The relationship between prepartum nutrition and subsequent breeding season pregnancy rates is well established (Wiltbank et al., 1970; Bellows and Short, 1978; Short et al., 1990). This relationship is especially critical for primiparous heifers and young cows due to the added nutrient requirement of their own growth, resulting in a higher risk of reproductive failure compared with older cows (Meek et al., 1999).

Providing supplemental protein to beef cattle grazing low quality forages has been reported to increase forage intake, improve cow BW gain, and may increase pregnancy rate (reviewed in DelCurto et al., 2000). However, results vary based on protein source, degradability, and physiological status (Wiley et al., 1991; Rusche et al., 1993; Triplett et al., 1995; Patterson et al., 2003). Therefore, objectives of the current study were to determine the effect of supplemental protein source on ADG, feed intake, calf birth BW, and subsequent pregnancy rate in pregnant beef heifers.

Materials and Methods

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all procedures and facilities used in this experiment.

Pregnant heifer management. A 3-yr study was conducted at the West Central Research and Extension Center (**WCREC**), North Platte. Crossbred, Angus-based, AI-pregnant heifers (yr 1 n = 38, yr 2 n = 40, yr 3 n = 36) were stratified by BW (450 ± 10 kg) and placed in a Calan Broadbent individual feeding system at approximately d 142 of gestation. Heifers were allowed approximately 25 d to adapt to the individual feeding system followed by an 84 d feeding trial. Heifers were offered ad libitum grass hay (8 to 11% CP, DM basis) and either no supplement (**CON**), 0.83 kg/d distillers based supplement (**HI**), or 0.83 kg/d dried corn gluten based supplement (**LO**, Table 1). Supplements were formulated to be isocaloric and isonitrogenous, and equal in lipid content; but differ in rumen undegradable protein. Feed offered was recorded daily and refusals removed and weighed weekly. Residual feed intake (**RFI**) was calculated as the actual DMI minus predicted DMI, with DMI calculated based on NE values of the feed to account for different energy levels of the supplement compared with the control diet.

Post-calving management. After calving, cows and calves remained at WCREC through AI. Prior to the breeding season, blood samples were collected 10 d apart via coccygeal venipuncture to determine plasma progesterone concentration. Plasma progesterone concentration was determined through direct solid phase RIA (Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA). Cows with plasma progesterone concentrations >1.0 ng/mL were considered to have resumed estrus.

Estrus was synchronized utilizing a controlled internal drug release (**CIDR**; Pfizer Animal Health, New York, NY) protocol, with cows receiving 100 μg; i.m.

GnRH (Fertagyl, Intervet Inc., Millsboro, DE) and CIDR insert on d 0. Seven d later, CIDR was removed and a single injection of $PGF_{2\alpha}$ (25 mg; i.m.; Lutylase, Pfizer Animal Health, New York, NY) administered followed by GnRH and AI approximately 60 h later. Following AI, cows and calves were transported 43 km to a commercial ranch in the Nebraska Sandhills for summer grazing. A single bull was placed with heifers approximately 10 d after AI for 60 d. Cows and calves were returned to WCREC prior to weaning for final pregnancy diagnosis. Following weaning, all pregnant 2 yr old cows grazed corn residue and received 0.45 kg/d (32% CP, DM basis) distillers based supplement.

Statistical analysis. Heifers were offered hay and supplement on an individual basis during the experimental period; therefore, heifer was considered the experimental unit and diet the treatment. The statistical model included treatment as the fixed effect with pen and year as random effects. Calf sire and gender were included in the model for calving data. Data were analyzed using PROC MIXED and PROC GLIMMIX of SAS for categorical and binomial data, respectively. Regression analysis utilizing PROC REG of SAS was used to determine the relationship between DMI, diet, and week of gestation. There was no intake × diet interaction (P = 0.62); thus, regression was utilized to determine the relationship of DMI and week of gestation. Data were considered significant if $P \leq 0.05$.

Results and Discussion

Individual feeding results (Table 2). Heifers not receiving supplement tended ($P = 0.09$) to consume less total DM than either supplement treatment. Similarly, NE DMI was less ($P < 0.01$) for CON heifers (4.98 ± 0.23 kg) compared with HI or LO heifers (5.43 and 5.35 ± 0.23 kg, respectively). However, forage-only DMI was greater ($P < 0.01$) for CON heifers (9.94 ± 0.12 kg) compared with HI or LO heifers (8.50 and 8.34 ± 0.12 kg, respectively).

Forage intake declines when CP values are below 7% (Mathis, 2000). Providing supplemental protein when cattle are grazing or consuming low quality forage may increase forage DMI (DelCurto et al., 2000). In the present study, forage CP content was greater than 7% and subsequently protein supplement replaced forage intake in HI and LO heifers. These data agree with Olson et al. (1998) reporting decreased total OM intake in heifers supplemented RUP during late gestation. Similarly, Loy et al. (2007) reported heifers provided chopped grass hay (8.2% CP) and 0.4% BW/d of either dry-rolled corn or dried distillers grain supplement had reduced ($P < 0.01$) hay DMI compared to nonsupplemented heifers. Furthermore, forage DMI was similar ($P = 0.45$) regardless of supplement type, comparable to findings in the current study. However, Strauch et al. (2001) reported heifers offered stockpiled tall fescue forage (11.7% CP) had increased prepartum forage DMI when heifers were supplemented an additional 84 g/d (106 vs. 190 g/d) RUP for 64 d prior to calving compared with control heifers.

Heifers receiving no supplement had less ADG (0.59 ± 0.14 kg) than either HI (0.82 ± 0.14 kg) or LO (0.78 ± 0.14 kg, $P < 0.01$) heifers, resulting in reduced ($P < 0.01$) BW (501 ± 9 kg) compared with HI heifers (519 ± 9 kg) at the end of the trial. The differences in diet nutrient density resulted in a greater ($P < 0.01$) NE intake for the HI and LO heifers compared with the CON heifers. Although DMI tended to be greater for HI compared with CON heifers, G:F was greater ($P < 0.01$) for HI compared with CON heifers. The increase in G:F can be attributed to improved ADG for HI heifers, which was approximately 1.5 times greater than CON heifers. However, CON heifers had increased RFI based on diet NE ($P < 0.01$) compared with HI and LO heifers, whereas RFI between supplement groups was similar ($P = 0.97$). Nichols (2010) reported providing heifers either 102 or 119% MP during the second and third trimesters of gestation had no effect on RFI; however, both treatments exceeded heifer MP requirements. These data are similar to the current study reporting no difference in RFI for HI or LO heifers, with MP requirement also being exceeded.

Dry matter intake was greatest at gestation wk 28 (10.06 ± 0.48 kg/d) and decreased ($P = 0.01$) as week of gestation increased through the remainder of the feeding period (wk 38). Vanzant et al. (1991) reported similar grazing time for pregnant and non-pregnant heifers approximately 55 d prior to parturition, but reduced grazing time for pregnant heifers approximately 12 d prior to parturition. Interestingly, OM intake was similar for pregnant heifers 55 and 12 d prior to parturition. Conversely, Forbes (1968) reported rumen capacity is reduced due to increased uterine volume during late gestation due to rapid fetal growth. Thus, increasing nutrient density in late gestation diets to meet MP requirements is critical.

Calving and subsequent pregnancy results (Table 3). Julian birth date and gestation length were similar among treatments ($P > 0.36$). Calf birth BW, calving ease, and calf vigor did not differ ($P > 0.14$) among treatments. Subsequent postnatal calf performance through slaughter is reported elsewhere (Summers, 2012).

At pre-breeding, CON heifers had decreased ($P < 0.03$) BW compared with LO heifers. However, prepartum supplementation did not ($P = 0.51$) influence the proportion of heifers resuming luteal activity prior to the breeding season. Cow BW was similar ($P = 0.48$) among treatments at pregnancy diagnosis. The proportion of cows pregnant to AI and final pregnancy rate was similar ($P \geq 0.22$) among treatments.

Cows were synchronized utilizing a CIDR estrus synchronization protocol. Lucy et al. (2001) reported CIDR increased the proportion of anestrous cows detected in estrous within the first 3 d of the breeding season compared with $PGF_{2\alpha}$-treated or control cows. It is possible the synchronization protocol used in the current study increased synchronization response and subsequent pregnancy rates to AI given the relatively low percentage of cows resuming estrus prior to synchronization. Regardless, prepartum supplement treatment did not affect resumption of estrus prior to CIDR insertion.

The impact of late gestation nutrition on subsequent pregnancy rate has been inconclusive (reviewed by DelCurto et al., 2000). Rusche et al. (1993) reported

conception rate was similar for primiparous heifers supplied either 100 or 150% NRC recommendation for CP in diets containing either low or high RUP. Patterson et al. (2003) reported increased pregnancy rates for heifers supplemented with RUP during late gestation to balance MP requirements compared to heifers supplemented to balance CP requirements. Also, Engel et al. (2008) reported providing heifers a diet of hay and distillers grains with solubles during late gestation improved pregnancy rate 10 percentage points ($P = 0.06$) compared with heifers offered hay and soybean hulls. In both studies (Patterson et al., 2003; Engel et al., 2008), pregnancy rates were decreased in heifers offered diets deficient in MP during late gestation. Nichols (2010) reported similar pregnancy rates for heifers supplied either 102 or 119% of MP requirements; suggesting excess MP does not improve pregnancy rates. Similarly in the present study, all diets supplied excess MP (Summers, 2012), which may explain the lack of treatment effects on pregnancy rates.

Implications

Previous reports have indicated the importance of prepartum nutrition on subsequent pregnancy rates and longevity. In the current experiment, protein supplementation increased ADG in pregnant heifers; however, calf birth BW, resumption of estrus, and subsequent pregnancy rates were similar, regardless of supplementation or supplemental protein source. All diets in the current study were balanced for or exceeded MP requirements, which may explain the lack of treatment effects. Future studies restricting heifer MP intake during mid- to late gestation are warranted to determine the impact protein source and level may have on feed intake, ADG, and reproductive efficiency.

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Table 1. Composition of supplements offered to heifers during feeding trial

	% DM	
Ingredient, %	High[1]	Low[2]
DDGS[3]	99.0	-
CGF[4]	-	72.4
Corn germ	-	24.5
Urea	-	2.1
Trace minerals and vitamins	1.0	1.0
Nutrient Analysis[5], %		
CP	28.2	28.1
RUP, % CP	59.0	34.0
TDN	79.4	77.3
Crude fat	11.9	11.9

[1]Heifers offered 0.82 kg/d (DM) distillers grain based supplement.
[2]Heifers offered 0.82 kg/d (DM) dried corn gluten feed based supplement.
[3]Dried distillers grains with solubles.
[4]Dried corn gluten feed.
[5]Wet chemistry, Ward Laboratories Inc., Kearney, NE; RUP based on NRC (1996).

Table 2. Impact of supplemental protein source on ADG, feed intake, and feed efficiency in pregnant beef heifers

Item	No supplement[1]	High RUP[2]	Low RUP[3]	SEM	*P*-value
Initial BW, kg	452	451	448	10	0.74
Final BW, kg	501[a]	519[b]	513[a,b]	9	<0.01
DMI, kg	9.94	10.32	10.16	0.12	0.09
Forage DMI, kg	9.94[a]	8.50[b]	8.34[b]	0.12	<0.01
NE DMI, kg	4.98[a]	5.43[b]	5.35[b]	0.23	<0.01
ADG, kg	0.59[a]	0.82[b]	0.78[b]	0.14	<0.01
RFI, DMI, kg	-0.017	0.008	-0.019	0.171	0.98
RFI, NE, kg	-0.211[a]	0.083[b]	0.064[b]	0.295	<0.01
G:F kg gain/kg	0.059[a]	0.078[b]	0.076[b]	0.014	<0.01

[1]Offered ad libitum grass hay (8 to 11% CP, DM basis) and no supplement.
[2]Offered ad libitum grass hay (8 to 11% CP, DM basis) and 0.83 kg/d (DM; 28% CP) distillers grain based supplement.
[3]Offered ad libitum grass hay (8 to 11% CP, DM basis) and 0.83 kg/d (DM; 28% CP) dried corn gluten feed based supplement.
[a,b]Within each row, means without common superscripts differ ($P < 0.05$).

Table 3. Impact of supplemental protein source on subsequent cow and calf characteristics

Item	No supplement[1]	High RUP[2]	Low RUP[3]	SEM	*P*-value
Julian birth date, d	60	60	62	1	0.36
Gestation length, d	276	276	277	1	0.88
1st calf birth BW, kg	33	33	33	1	0.99
Calving ease[4]	1.40	1.39	1.53	0.13	0.70
Calf vigor[5]	1.41	1.46	1.89	0.19	0.14
Resumption of estrus, %	25	27	37	11	0.51
Prebreeding BW, kg	445[a]	458[b]	460[b]	13	0.03
Pregnancy diagnosis BW, kg	483	488	493	12	0.48
Retention rate, %[6]	92	90	82	5	0.35
AI pregnancy rate, %	59	56	64	10	0.80
Overall pregnancy rate, %	90	91	79	12	0.22
Second calf Julian birth date, d	68	72	64	4	0.19
AI to parturition, d	290	294	286	4	0.20
Calved first 21-d, %	73	65	87	9	0.20

[1]Offered ad libitum grass hay (8 to 11% CP, DM basis) and no supplement.
[2]Offered ad libitum grass hay (8 to 11% CP, DM basis) and 0.83 kg/d (DM; 28% CP) distillers grain based supplement.
[3]Offered ad libitum grass hay (8 to 11% CP, DM basis) and 0.83 kg/d (DM; 28% CP) dried corn gluten feed based supplement.
[4]Calving ease scoring system: 1 = no assistance, 2 = easy pull, 3 = mechanical pull, 4 = hard mechanical pull, 5 = Caesarean section.
[5]Calf vigor scoring system: 1= nursed immediately; 2= nursed on own, took some time; 3= required some assistance to suckle; 4= died shortly after birth; 5= dead on arrival.
[6]Proportion of cows remaining at the beginning of the second breeding season.
[a,b]Within each row, means without common superscripts differ ($P < 0.05$).

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WHAT IS THE EFFECT OF SHEEP GRAZING FOR COVER CROP TERMINATION ON ASSOCIATED BIODIVERSITY?

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ABSTRACT: Targeted sheep grazing of cover-crops could potentially benefit agriculture by enhancing nutrient cycling, soil conservation, and pest management. Because grazing represents an ecological filter, it is important to understand the impacts this practice may have on the associated biodiversity of agroecosystems. We compared the effects of sheep grazing and mowing for cover-crop termination on plant and carabid beetle (Coleoptera: Carabidae) community structure at Towne's Harvest Farm in Bozeman, MT. Metrics for plant communities included plant diversity, weed biomass, and cover-crop biomass. Metrics for carabid beetle communities, which are beneficial generalist predators in agroecosystems, included activity-density, species richness, and diversity. In six 10 m × 15 m plots, we seeded a cover crop of buckwheat (*Fagopyrum esculentum* Moench), beet (*Beta vulgaris* L.), sweetclover [*Melilotus officianalis* (L.) Lam.], and pea (*Pisum sativum* L.). We allowed the sheep to graze until plots had ≥ 90% of the biomass removed, which we determined by visual inspection. For the mowing treatment, we mowed plots using a tractor mowing deck and ensured that vegetation within each plot was homogeneously cut. Plant biomass samples were estimated prior to cover-crop termination and again one month post termination. Carabid beetles activity-density was assessed throughout the growing season using pitfall traps. We did not detect any significant treatment differences in plant biomass and diversity ($P > 0.10$) and carabid beetle activity-density and diversity ($P > 0.37$). Our results suggest that sheep grazing for cover crop termination has a similar effects on associated biodiversity as that of mowing. Thus, farmers choosing to implement sheep grazing for cover crop termination should not experience adverse changes in plant community composition or carabid beetle assemblages.

Introduction

Agricultural intensification has resulted in homogenized landscapes and decreased ecosystem complexity (Foley et al., 2005). Decreased ecosystem complexity may adversely alter important ecological properties for crop cultivation including soil structure, hydrology, nutrient cycling, plant community structure, and insect predator-prey dynamics (Altieri, 1999; Benton et al., 2003; Shennan, 2008). Ecologically-based agricultural practices may mitigate some of these deleterious changes to ecosystem function and structure caused by agricultural intensification. For example Mader (2002), found that organic practices increased activity-density and diversity of a variety of beneficial soil organisms compared with those of conventional agricultural practices. The increased soil organism activity observed in diversified agricultural systems was associated with an enhancement in soil resource utilization efficiency as well as numerous metrics of soil fertility. Despite their potential benefits, farming practices pose several challenges for crop production. Unlike their conventional counterparts, farmers interested in sustainable management practices aim at reducing their reliance on off-farm synthetic inputs while taking advantage of ecologically-based processes to cope with oscillating levels of pest pressures and nutrient availability. Thus, alternative land management practices are needed in order to overcome impediments to production such as pestiferous arthropods, weedy plant species and soil nutrient levels.

Cover cropping is widely regarded as a beneficial practice for improving soil quality. This practice can increase the N capital by *Rhizobia*-mediated N-fixation when Fabaceous plants are included in the cover crop. Cover cropping can also increase the water holding capacity, the cation exchange capacity, and decrease the penetration resistance of the soil (Dabney et al., 2001). Cover cropping may also have beneficial ecological impacts such as suppressing weed population and attracting beneficial arthropods (Tillman et al., 2012). However, one major drawback of cover cropping for farmers is that it requires that they do not grow a cash crop the year that they grow a cover crop (Thiessen Martens and Entz, 2011). This often renders cover cropping economical unfeasible.

Integrating livestock may provide an alternate source of income for farmers and thus make cover cropping economically feasible. In addition, targeted livestock grazing for cover crop termination is a promising agricultural practice that may help farmers reduce their reliance on off-farm synthetic inputs (Sulc and Tracy, 2007; Franzluebbers, 2007; Thiessen Martens and Entz, 2011). However, like mowing, livestock grazing represents an ecological filter for plant and arthropod communities. Before implementing such a practice, it is critical to understand how integrating livestock grazing for cover crop termination will affect associated biodiversity. As part of a larger study investigating the ecological ramifications of integrating sheep grazing onto farming systems, we

compared the effects of sheep grazing for cover crop termination with those of tractor mowing on plant and carabid beetle community structure. Specifically, our goal was to determine whether the effects of sheep grazing on the associated biodiversity are comparable with, more beneficial than, or more detrimental than those of mowing in organic agroecosystems.

Methods

Experimental Design

Our experiment was designed as a single factor, completely-randomized experiment with two treatment-levels (sheep-grazed and mowed) and three replicates per treatment-level. Each plot was a 10 m × 15 m rectangle. In June 2012, we cultivated the soil in all plots and seeded a cover crop consisting of buckwheat (*Fagopyrum esculentum* Moench), beet (*Beta vulgaris* L.), sweet clover [*Melilotus officianalis* (L.) Lam.], and pea (*Pisum sativum* L.)

Between August 3, 2012 and August 7 2012 we terminated the cover crops before they fruited. We tractor mowed half of our plots to terminate the cover crops. For our sheep-grazed plots, we set up temporary electrical fences charged between 3500V – 6000V, stocked each plot with 6 – 11 ewes and allowed those sheep to graze *ad libitum* until the cover crop appeared ≥90% removed.

Plant Community Data

To estimate plant community structure, we collected aboveground plant biomass from four randomly placed $0.4m^2$ quadrats in each plot. We cut all plant material flush with the soil surface. To avoid pseudoreplication, we separated each species and concatenated the total biomass for each species from each of the four quadrats. We collected biomass prior to cover crop termination in late July 2012 and again in mid September 2012, after cover crop termination and regrowth, but prior to fall senescence. We dried all samples to constant mass at 60°C and weighed them to the nearest 0.1 g..

Carabid Beetle Community Data

To characterize the carabid beetle community, we measured activity-density, a proxy measure for species abundance and community structure, by pitfall trapping. The number of beetles caught in a pitfalll trap depends on both how many beetles are present and how much the move through that ecosystem (Thomas et al., 1998). Thus, activity and density are inherently confounded using this method, and quantitative results from pitfall trapping are referred to as activity-density (Holland et al., 2002).

To estimate activity-density, we placed three pitfall traps in the center of the each one of our six experimental plots. To construct the pitfall traps, we dug approximately 20 – 30 cm deep X 10 cm wide holes with a post-hole digger and placed two stacked 16 oz plastic cups (Solo Cup Company, Lake Forest, IL) in each of those holes. We backfilled the pitfall trap holes until the mouth of the top cup was flushed with the soil surface and filled the top cup of the pitfall trap approximately one-third full with propylene glycol (Arctic Ban®, Camco Manufacturing Inc., Greensboro, NC) as a killing agent. We covered each pitfall trap with a rain cover constructed with 25cm–diameter clear plastic plates hold to the ground with three equally spaced 10cm bolts. Each rain cover had at least 2cm between the soil surface and the rim of the clear plastic plates to avoid interfering with ground dwelling arthropod activity.

We collected all arthropods caught in the pitfall traps between May 25, 2012 and June 1, 2012 to determine the carabid beetle community prior to soil cultivation and cover crop seeding. Additionally, each week between June 22, 2012 and July 27, 2012 for the pre-treatment period and August 16, 2012 and October 5, 2012 for the post-treatment period, we collected all arthropods caught in the pitfall traps. Due to an error, the pitfall traps were not collected on July 21, 2012, and therefore the samples from July 27, 2012 represented two weeks of collection. To correct for this error, we calculated the mean daily catch rate for each capture period. We placed the catch into a 9.5 × 18 cm plastic bag (Whirl-Pak®, Nasco Inc., Fort Atkinson, WI) and refilled the pitfall traps with antifreeze. To avoid psuedoreplication, we combined the three pitfall traps within each plot into the same plastic bag, stored them in a freezer, and later transferred the samples into 70% ethanol by volume. We identified all carabid beetles to species in each sample following Lindroth (1968).

Data Analysis

We compared total dry plant biomass, cover crop biomass, weed biomass, and species diversity between grazed and mowed plots. To measure diversity, we used the Shannon-Weaver index (H´):

$$H' = -\sum_{i=1}^{S} p_i \ln p_i \qquad (1)$$

where S is the species richness of a community, i indexes the species in that community, p_i is the proportion of individuals in a community of species i, (Peet, 1974). Because the Shannon-Weaver index favors rare species (Hector et al., 2002), we also calculated Simpson's diversity index ($1 - D$):

$$H' = -\sum_{i=1}^{S} p_i \ln p_i \qquad (2)$$

where S is the species richness in a community, i indexes the species that comprise that community, n_i is the number of individuals in species i, N is the total number of individuals in a community and D is the Simpson dominance (Peet, 1974). We used a two-independent sample permutation test with 1000 iterations to compare

these metric between mowed and grazed plots prior to cover crop termination and after cover crop termination.

For the carabid beetle data, we calculated species richness, total activity density, and species diversity (indexed with both the Shannon's H and Simpson's 1-D). To compare carabid beetle community metrics, we used a repeated measures ANOVA with date as a fixed effect and plot as a random effect. For all statistical tests, we considered results significant when $p < 0.05$. All data are reported as mean ± S.E.

Results

Plant Community Data

Prior to cover crop termination, weed species biomass in the plots to be grazed was equivalent from that sampled in the plots to be mowed ($P = 0.694$, two-sample permutation test, 286.7 ± 120.4 g m^{-1} and 235.8 ± 64.3 g m^{-1} in grazed and mowed plots, respectively). Similarly, cover crop biomass was not significantly different between the grazed and mowed treatments prior to cover crop termination ($P = 1.00$, two-sample permutation test, 191.5 ± 78.8 g m^{-1} compared with 151.5 ± 30.1 g m^{-1} in grazed and mowed plots, respectively). .

After we implemented our treatments, neither cover crop biomass nor weed biomass was significantly different between grazed and mowed plots (for cover crops: $P=0.10$; two-sample permutation test, 3.3 ± 1.3 g m^{-1} and 15.0 ± 5.4 g m^{-1} in grazed and the plots to be mowed plots, respectively). Similarly, there was no differences in weed biomass between treatments (P=0.90, two-sample permutation test, 105.8 ± 55.1 g m^{-1} 123.0 ± 31.2 g m^{-1}, in grazed and mowed plots, respectively).

Neither Shannon's H nor Simpson's 1 – D differed between grazed and mowed plots prior to cover crop termination (for H: P = 0.10, for 1 – D: P = 0.10; two-sample permutation test). For grazed plots, H was 1.23 ± 0.20 and 1 – D was 0.52 ± 1.1. For mowed plots, H was 1.67 ± 0.05 and 1 – D was 0.76 ± 0.01. Similarly, diversity did not differ between treatments following cover crop termination (for H: P = 0.10, for 1 – D: P = 0.10; two-sample permutation test). For grazed plots, post-treatment H was 0.82 ± 0.16 and 1 – D was 0.46 ± 0.08, whereas for mowed plots H was 1.06 ± 0.22 and 1 – D was 0.51 ± 0.11. We failed to detect differences in plant diversity between the pre- and post-treatment plant communities in the grazed plots (for H: P = 0.76, for 1 – D: P = 1.00; paired permutation test). Similarly, we did not detect any difference in diversity between pretreatment and post-treatment communities in mowed plots.

Carabid Beetle Community Data We found no treatment effects on carabid beetle activity-density at any point during the growing season (F = 0.27; df= 1, 4; P = 0.63). Prior to cover crop seeding, total carabid beetle activity-density was 5.95 ± 1.63 beetles d^{-1} in grazed plots and 6.00 ± 0.87 beetles d^{-1} in mowed plots. Total carabid beetle activity-density increased from seeding until termination for grazed plots. Activity-density was 5.52 ± 0.69 and 8.12 ± 0.79 beetles d^{-1} for the collection period immediately following seeding (Julian dates 172 – 179) and for the collection period immediate preceding termination (Julian dates 194 - 208), respectively. For mowed plots, however, carabid beetle activity-density increased following cover crop seeding, but declined during the collection period prior to cover crop termination. Activity-density was 5.14 ± 0.79 and 5.05 ± 0.77 beetles d^{-1} for the collection period immediately following seeding and the collection period immediately preceeding termination.. Following cover crop termination, activity-density declined for both treatments. For the grazed plots, activity-density was 4.75 ± 2.82 beetles d^{-1} in the collection period immediately following termination (Julian dates 229 - 235) and 0.10 ± 0.05 beetles d^{-1} for the final collection period (Julian dates 271-278).

Carabid beetle species diversity was variable throughout the season for both treatments and fluctuated similarly for both treatments and diversity indices. A repeated measures ANOVA found that there was no significant effect of treatment on diversity for either Shannon's H (F = 1.02 ; df = 1,4; P = 0.37) or for Simpson's D (F = 0.95; df = 1,4 ; P = 0.39). The maximum diversity occurred prior to seeding the cover crop. During this collection period (Julian dates 145 – 152) Shannon's H was 1.93 ± 0.08 and 1.72 ± 0.16 for grazed and mowed plots, respectively. Simpson's diversity was 0.83 ± 0.01 for grazed periods 0.78 ± 0.04 for mowed plots during the same period. During the period following seeding but prior to termination of the cover crop, diversity generally declined in both treatments. For grazed plots, Shannon's H reached a nadir of 0.69 ± 0.29 during the collection period between Julian dates 187 – 194. Simpson's diversity was 0.32 ± 0.16 during this period. For mowed plots, Shannon's H reached a nadir of 1.01 ± 0.46 during the collection period between Julian dates 194 – 208. Simpson's diversity was 0.45 ± 0.20 for the same period. Carabid beetle diversity rebounded immediately following cover crop termination, and oscillated for the remainder of the sampling season. During the collection period immediately following termination (Julian dates 229-236), H was 1.59 ± 0.21 and 1 – D was 0.76 ± 0.05 for the grazed plots, and for mowed plots these values were 1.62 ± 0.09 and 0.78 ± 0.22 for H and 1 – D, respectively. During the final collection period, between Julian dates 271 and 278, H was 0 and 0.58 ± 0.32, and 1 – D was 0.33 ± 0.33 and 0.37 ± 0.20 for grazed and mowed plots, respectively.

We collected 37 species of carabid beetles. The species with the greatest activity-density included *Pterostichus melanarius* Illiger, *Poecilus scitulus* Leconte, *Amara patruelis* (Dejean), *A. thoracica* (Hayward), *Harparlus amputatus* (Say), and *Bembidion rupicola* (Kirby). Prior to seeding, *B. rupicola***,** *Bradycellus congener* (Leconte), and *Stenolophus comma* (Fabricius). In grazed plots, the activity-densities of *B. rupicola*, *B. congener* and *S. comma* were 1.14 ± 0.22, 0.95 ± 0.38, and 1.38 ± 0.50 beetles d^{-1}, respectively, and in the mowed plots 1.86 ± 0.46, 1.00 ±

0.38 and 1.00 ± 0.22 beetles d^{-1}, respectively. Following seeding, *P. melanarius*, *P. scitulus* and *A. patruelis* became the most frequently captured species. *P. melanarius* in particular dominated the carabid community during collection periods while the cover crop was growing. *P. melanarius* reached a maximum activity-density of 6.90 ± 3.15 beetles d^{-1} in grazed plots and 4.04 ± 3.05 beetles d^{-1} during the collection period between Julian dates 187 – 194. However, following cover crop termination, *P. melanarius* activity-density declined preciptiously. In the first collection period following termination (Julian dates 229 – 236), its activity-density was 2.00 ± 1.51 beetles d^{-1} in grazed plots and 1.54 ± 0.61 beetles d^{-1} in mowed plots. We did not collect any *P. melanarius* after Julian date 264 for either treatment.

Discussion

We did not detect any differences among the plant communities in grazed and mowed plots as both treatments reduced cover crop biomass similarly. There are two possible explanations for this result. First, most of the cover crops that we planted are likely to be highly palatable for sheep. Thus, the sheep were unlikely to avoid systematically any of the cover crops. Second, the farm's irrigation line ran through the southern half of our experimental site and half of our plots received more water those in the northern half of our experimental site. The southern plots had more biomass than did the northern plot, and thus made our data highly variable within treatments. Similarly, There was no difference between weed biomass in grazed mowed plots. While the lack of a difference between mowed and grazed plot may be a statistical artifact due to high variability within treatment, a type II error resulting from too few degrees of freedom, the similar mean values of these parameters suggest that both methods of cover crop termination have the same effects on the plant community.. However, we noticed that the sheep systematically avoided redroot pigweed *Amaranthus retroflexus* (L.) and common mallow *Malva neglecta* Wallr. Similarly, the mowing implement often missed *M. neglecta* because it did not reach the height of the blade. *A. retroflexus* regrew quickly following mowing, often because the blade failed to severe stems completely. These systematic biases of grazing and mowing against *A. retroflexus* and *M. neglecta* were apparently similar enough to avoid differentially reducing weed biomass.

Grazing and mowing both had little effect on changes in cover crop plant diversity. Plant diversity was similar in grazed plots to that in mowed plots both before and after cover crop termination. Furthermore, we found that neither the Shannon-Weaver diversity index nor Simpson's diversity index changed for plant communities using either cover crop termination method.

While the activity-density of carabid beetles fluctuated throughout the season, the activity-density and diversity of carabid beetles did not differ between treatments. Activity-density generally increased while the cover crop was actively growing and then declined following termination. The increase in total activity-density while the cover crop was growing is likely due to an influx of *P. melanarius*, which we collected far more frequently than any other carabid species during this period. Conversely, the decline in total carabid activity-density following cover crop termination is likely a result of *P. melanarius* migrating out of our plots. As noted in the results section, we collected progressively fewer members of this species in post-treatment collection periods.

The decline in diversity following cover crop seeding is also likely a result of a precipitous influx of *P melanarius* with increasing canopy cover, which, in turn, resulted in an increased dominance of this species. Following cover crop termination, *P. melanarius* activity-density declined while that for other species such as *A. thoracica*, *A. patruelis* and *H. amputatus* increased. The decline in *P. melanarius* dominance likely explains the concomitant increase in diversity immediately following cover crop termination. However, despite fluctuations throughout the season, carabid beetle diversity did not differ at any point between treatments for either diversity index. These results suggest that mowing and grazing for cover crop termination have similar effects on carabid beetle activity-density and community structure. If the Carabidae is an effective bioindicator taxon in our system, these results may suggest that both methods of cover crop termination have similar effects on the associated arthropod community in similar agroecosystems.

In conclusion, our results suggest that sheep-grazing and mowing for cover crop termination affect the associated biodiversity of organic agroecosystems similarly. These results suggest that sheep grazing and mowing act as similar ecological filters of plant and carabid beetle diversity. Thus, farmers choosing to implement sheep grazing *in leiu* of mowing for cover crop termination should not experience major changes to the plant or carabid beetle community. Given its potential to generate income for farmers during season when cover crops are planted, sheep grazing may make the practice of cover cropping economically viable and ecologically sound.

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EVALUATION OF MOSQUITO RESPONSES TO TOPICALLY APPLIED PYRETHROID INSECTICIDES TO SHEEP

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ABSTRACT: A rise in the incidence of mosquito transmitted Cache Valley virus (CVV) in lambs in 2011 prompted a study to evaluate on-animal pyrethroid insecticides to reduce mosquito attacks on sheep. Using enclosure traps for 1 night per wk. for 6 wks., we compared engorgement rates of mosquitoes given the opportunity to feed on untreated sheep and sheep treated with 1 Python insecticide ear tag per animal or 2 synergized permethrin body spray treatments. During the 6 wk. study, 18,920 mosquitoes were collected in the animal-baited enclosure traps. Thirteen species were identified from these collections with the floodwater species *Aedes increpitus* and *Ae. idahoensis* comprising 68% of the total. Potential CVV vector species, comprising 25% of the samples, included *Ae. vexans, Ae. dorsalis, Culex tarsalis* and *Culiseta inornata*. Traps baited with untreated sheep collected 9,701 mosquitoes with 65% of these engorged. Traps baited with sheep treated with Python ear tags or permethrin spray collected 4,034 and 4,555, respectively, with engorgement rates of 23% and 35%. Over the six week trapping period, blood feeding on ear tagged sheep was significantly ($p<0.03$) reduced by as much as 90% compared to the untreated sheep, and protection lasted 4 wk. or longer. Permethrin spray treatments were most effective within 24 h after application and provided better protection against *Ae. dorsalis* than the Python tag ($p<0.05$) . Effectiveness of the permethrin spray diminished 1 wk after the second application was made ($p< 0.03$). The effect of these treatments appeared to be repellency because negligible mosquito mortality was observed at the time of collection. Further evaluation of these insecticides under conditions of natural exposure to a mosquito-borne pathogen is warranted.

Keywords: Cache Valley virus, insecticide, mosquito, pyrethroid, sheep

Introduction

Sheep producers in several states in the upper Midwest and northern Great Plains experienced an increase in the number of lambs that were stillborn or malformed during the 2011 lambing season. Several producers in central North Dakota estimated >20% lamb losses (R. Redden, NDSU, pers. com). Reproductive defects in sheep are influenced by a number of factors including ram fertility, nutritional status at breeding or infectious diseases (e.g., bluetongue, Border Disease). In this case, Cache Valley virus (CVV) was confirmed by state veterinary diagnostic laboratories in ND, SD, MN, and WI as an agent responsible for the increased morbidity rate.

A *Bunyavirus*, CVV was first detected in North America in 1956 in pools of *Culiseta inornata* Williston collected from northern Utah (Holden and Hess 1959). The virus has since been isolated from 30 mosquito species in six genera (Moore and Mitchell 1997). CVV is endemic in several regions of the continental United States and central provinces of Canada (Calisher et al. 1986). It has been detected in many species of domestic livestock and wildlife including sheep, swine, goats, cattle, and deer (Calisher et al. 1986, Campbell et al. 2006).

In domestic sheep, the majority of CVV infections are subclinical except when transmission occurs soon after ewes are bred. During the first 45 days of gestation, the virus may cross the placenta and infect the fetus, resulting in fetal loss, stillbirth, or severe muscular and neurological deformities of neonates (Edwards et al. 1989). An infection 45 days post-conception does not lead to significant disease in the fetus (de la Concha-Bermejillo 2003).

Management options to reduce the risk of CVV fetal infections in domestic sheep include breeding ewes in the late fall or early winter after mosquitoes subside and control of both immature and adult mosquitos. Another potential strategy is to topically treat sheep with an insecticide to repel or interrupt insect blood feeding.

Results from studies evaluating on-animal treatments on cattle are varied. A single application of a permethrin pour-on (Brute®, 10% permethrin) or whole body spray (Gardstar®, 0.05% permethrin) significantly reduced blood feeding on yearling steers by the floodwater mosquitoes *Aedes dorsalis* Meigen and *Ae. melanimon* Dyar at 4 d post-treatment but not

at 11 d post-treatment (Schmidtmann et al. 2001). The Python® ear tag, containing zeta cypermethrin and piperonyl butoxide, reduced *Ae. dorsalis* and *Ae. melanimon* blood feeding on yearling heifers and steers by nearly 80% at 4 wk. post-application (Lloyd et al. 2002). Loftin et al. (1996) reported reduced engorgement on yearling Hereford steers by *Ae. vexans* and *Psorophora confinnis* (Lynch Arrabalzaga) at one month by the Saber™ (10% cyhalothrin) and Terminator™ (20% diazinon) ear tags. Although feeding suppression was lower than what was reported by Lloyd et al. 2002.

Sheep and wool production remains an important livestock enterprise in the northern Great Plains and Rocky Mountain states. Many of the mosquito species from which CVV has been isolated in North America are ubiquitous to this region. Thus, a need exists to evaluate strategies to control hematophagous insects, both potential vector and nuisance species, on sheep. We evaluated two pyrethroid insecticides, applied to sheep as an ear tag or body spray, on the blood feeding success of mosquito species common to the intermountain west.

Materials and Methods

The study was conducted from 6 July – 10 August, 2011 on an irrigated hay and grain farm near Toston, MT (N 46°09.993', W111°34.156', elev. 1,243 m). The land is flood-irrigated for hay production and is inundated by overflow from several streams during runoff in May and June. This area produces floodwater mosquitoes *Ae. increpitus, Ae. dorsalis* and *Ae. vexans* and permanent pool species including *Culex tarsalis* and *Culiseta inornata* (G.D. Johnson, unpublished data).

Experimental animals were Rambouillet lambs approximately 4 months of age, averaging 22.7 kg with an average wool length of 5 cm. The sheep were randomly assigned to one of three treatments. Three animals each received one Python insecticide ear tag; three animals were each sprayed with permethrin pour-on (YT-1P086, Lot # P01-5/28/09); and three animals received no treatment. These insecticides were selected because they have 24c registrations in MT and WY. Python tags (9.5 g per tag), containing 10% zeta-cypermethrin and 20% PBO, were applied on June 22, 2011 two weeks before the study began to allow release and distribution of insecticide. Permethrin oil-based pour-on, containing 2.5% permethrin and 2.5% PBO, was applied at a rate of 12 ml per animal through an atomized spray to the sides, back, chest, head and belly of each animal. Permethrin spray treatments were applied on 6 July and again on 13 July 8 h prior to placing sheep in traps to allow for drying. The second treatment was deemed necessary because the mosquitoes were feeding on the head, ears, muzzle and throat more so than the belly. After application of treatments, the treated groups were held in pens separated by two fences, to eliminate any potential for cross treatment contamination, for the duration of the study. Treated groups were also kept separate while being transported in a livestock trailer to and from the study site. Use of sheep for this research was approved by Montana State University Animal Care and Use Committee (2011-AA03).

Individual sheep were placed in one of nine stable traps (1.5 m X 1.8 m X 1.2 m) (H X L X W) constructed of wood with three metal gable vents (0.3 m^2) per side and a 0.3 m X 0.46 m gable vent on one end to allow host-seeking mosquitoes to enter. Traps, covered with fiberglass insect screen, were located in a 100 m X 40 m field adjacent to livestock corrals (no livestock were present during the study) and an equipment shed. Sheep were restrained in welded wire livestock panels (0.9 m X 0.9 m X 0.6 m) (H X L X W) but had sufficient space to turn around and lay down; alfalfa pellets and water were provided ad libitum.

Each treatment was replicated three times with each replicate consisting of one ear tagged animal, one animal sprayed with permethrin, and one untreated animal. Traps in a replicate were oriented in a north-south direction 10 m apart; replicates were 30 m apart. Within a replicate, the untreated and two treated sheep were randomly allocated to individual traps on the first trapping date and rotated to a different trap on subsequent trapping nights. Sheep were placed in traps one night per week at 1700 hr. and removed the next morning around 0800 hr. Prior to removing the sheep, vents were covered with screen and mosquitoes were aspirated from the inside of the traps using battery-operated aspirators (BioQuip Products Inc., Rancho Dominguez, CA). The aspirator catch bottles with mosquitoes were placed in a cooler with dry ice and transported to the lab for counting, identification to species, and determination of feeding status (non-engorged or engorged). Engorgement was determined by the expansion and coloration of the abdomen. Partially engorged individuals were classified as engorged.

The effectiveness of treatment was measured by suppression of blood feeding using Proc MIXED (Littell et al. 2002) with significance determined at $p \leq 0.05$.

Results

A total of 18,290 mosquitoes were collected from the sheep baited traps during the seven wk. study. Over half (9,701) of these were aspirated from traps holding untreated sheep. Mosquitoes collected from ear tag and spray treatments totaled 4,034 and 4,555,

respectively. Thirteen species were identified from these collections. *Aedes increpitus* (Dyar) and *Ae. idahoensis* (Theobald) comprised 68% of the total. Potential CVV vector species, comprising 25% of the samples, included *Ae. vexans, Ae. dorsalis, Cx. tarsalis* and *Cs. inornata*.

Approximately 65% of the total mosquitoes collected from traps baited with untreated sheep were engorged, which differed from 23% and 35% engorgement rates recorded in the ear tag and spray treatments, respectively (p<0.01). However, the total number of non-engorged mosquitoes between treatments did not differ (p=0.94). *Aedes dorsalis* had the lowest engorgement rate at 19% while *Ae. spencerii* (Theobald) and *Ae. increpitus* had the highest at 62% and 55%, respectively. During the collection process, knocked down or intoxicated mosquitoes (Mullens et al. 2000) were not observed, although a few dead mosquitoes were noted.

The four most abundant species collected in the animal traps were further analyzed for their response to treatments (Table 1). The mean number of engorged *Ae. increpitus* from the untreated (557.3), ear tag (52.7), and spray (137.0) treatments differed (p<0.01) on the first sample wk. This represents a 91% blood feeding suppression by ear tags and a 75% suppression by permethrin spray on wk. 1. Average wk. 2 captures of engorged *Ae. increpitus* from sheep in the ear tag and spray treatments did not differ (p=0.18). Compared to the untreated, blood feeding on wk. 2 was reduced 96% by the spray treatment and 91% by the ear tag. On sample weeks 3 thru 6, fewer (p<0.03) engorged *Ae. increpitus* were collected from the ear tagged than the spray-treated sheep. Python ear tag suppression of blood feeding by *Ae. increpitus* ranged from 61% to 95% (mean 88%) and the spray treatment reduced engorgement from 0 to 96% (mean 47%) for the seven wk study.

An average of 305.3 engorged *Ae. idahoensis* were collected from animals at wk. 1 with 58 engorged females collected from ear tagged sheep and 42.7 collected from spray-treated (Table 1). These treatment values represent reductions in blood feeding of 81% and 86%. At wk. 2, *Ae. idahoensis* mean catches of 111.3 (non-treated), 8.0 (ear tag), and 6.0 (spray-treated) differed (p<0.01) with the ear tag and spray treatments reducing blood feeding by 93% and 95%, respectively. The number of *Ae. idahoensis* captured in the traps dropped off sharply after wk. 2.

The average number of engorged *Ae. vexans* collected each week from untreated animals differed (p<0.02) from ear tag collections on weeks 2, 3, 4, 5, and 6 (Table 1). Differences (p<0.01) were also recorded between untreated animals and those treated with the spray on weeks 1, 2, 4, and 6. Additionally, on wk. 1, fewer (p<0.01) *Ae. vexans* were collected from sprayed than ear tagged sheep. Generally, during weeks 3 thru 6, sheep treated with the Python ear tag exhibited lower engorgement rates when compared to sheep treated with the spray. Effectiveness of the Python tag in reducing blood feeding ranged from 53% to 96% (mean 83%) and 10% to 98% (mean 68%) for the spray treatment for the 6 wk. study.

Discussion

Overall, the Python ear tag was more effective in reducing blood feeding by nuisance and potential vector species than the permethrin body spray. At one ear tag per animal, the maximum effectiveness was achieved at sample wk. 2 (four weeks post-tag application) where blood feeding was suppressed by 90% or greater. Lloyd et al. (2002) reported similar reductions in blood feeding by *Ae. dorsalis* and *Ae. melanimon* on yearling heifers and steers using two Python tags per animal at 4 wk. post-application.

The permethrin body spray was most effective at wk. 1 and 2, within 24 h after treatments were applied. A second application on wk. 2 concentrated on the head region, belly and axillary areas on the body where the wool was less dense or absent. As a result a higher percentage of blood feeding reduction was recorded on wk. 2 compared to wk. 1. Protection varied after wk. 2. Shemanchuk et al. (1991) used a permethrin low-pressure whole body spray which provided 70% or better protection from field populations of mosquitoes for 3 d. Shemanchuk suggested that even 3 days of protection could be acceptable, particularly in areas where single broods of mosquitoes occur.

Survival of blood engorged mosquitoes feeding on permethrin- or zeta-cypermethrin-treated cattle was measured in studies conducted by Schmidtmann et al. (2001) and Lloyd et al. (2002) but was not evaluated in this study. They reported no treatment-induced mortality of *Ae. Dorsalis* and *Ae. Melanimon* from either insecticide 24 h after their collection. Similarly, no mortality was observed in blood engorged black flies feeding on permethrin sprayed ponies (Schmidtmann et al. 2001). Based on these findings, they concluded that reduced bloodfeeding was the result of contact repellency. Loftin et al. (1996) recorded some mortality of *Ae. vexans* and *P. confinnis* that blood fed on steers treated with cyhalothrin or fenvalerate, but they also concluded that reduced blood feeding was due to a repellent response. In their study the restraining cages holding the steers were 30 cm from the inside wall of the stable traps, and they concluded any repellent effect was perceived from some distance away. The difference in the number of mosquitoes we collected in the traps holding pyrethroid-treated sheep compared to

untreated sheep also supports a repellent effect. And like Loftin et al., a mode of action other than direct contact must be involved because the restraining cages holding sheep were 25 cm from the inside of the stable traps.

These repellent observations are in contrast with studies that report substantial mortality of blood engorged diptera. In a caged bioassay study, McLaughlin et al. (1989) reported mortality up to 85% of *An. Quadrimaculatus* at one day post treatment of cattle with permethrin; mortality declined to 38% at 21 days post application.. Holbrook (1986) reported 93% mortality of *C. variipennis* exposed to hair samples from cattle treated with one fenvalerate ear tag per animal within 7 days post-treatment.

Python ear tags are economical at one tag per sheep and easy to apply. Tag retention was good and with the 9.5 g tag no ear problems were noticed. This method of control would work well for range flocks turned out to summer pasture where access to the animals is limited. The permethrin body spray was most effective immediately after treatment. In addition to the belly and breech area, it's important to provide coverage around the muzzle, throat, ears where mosquitoes were observed feeding. This method of application would work better on farm flocks that are easily accessible and can be re-treated more frequently.

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Table 1. Mean (± SD) number of non-engorged and engorged mosquito species collected and percent feeding suppression by treatment.

		Aedes increpitus			*Ae. idahoensis*			*Ae. dorsalis*			*Ae. vexans*		
Treatment	Wk[1]	Non-Engorged[2]	Engorged[2]	%[3]	Non-Engorged[2]	Engorged[2]	%[3]	Non-Engorged[2]	Engorged[2]	%[3]	Non-Engorged[2]	Engorged[2]	%[3]
Untreated	1	68.7 ± 55.2	557.3 ± 364.3a		200.3 ± 257.3	305.3 ± 258.4a		31.3 ± 25.2ab	13.7 ± 11.2ab		9.0 ± 4.4b	58.3 ± 30.0a	
Ear tag	1	101.7 ± 90.8	52.7 ± 19.7c	91	113 ± 27.9	58.0 ± 33.8b	81	70.0 ± 68.9a	17.3 ± 26.6a	0	42.3 ± 32.9a	27.3 ± 21.0a	53
Spray	1	147.0 ± 45.2	137.0 ± 80.6b	75	78.3 ± 54.2	42.7 ± 33.7b	86	13.3 ± 5.7b	2.7 ± 1.5b	80	14.0 ± 8.2b	6.7 ± 6.4b	89
Untreated	2	96.7 ± 56.6	190.7 ± 166.9a		68.0 ± 36.0	111.3 ± 125.0a		30.7 ± 35.5	11.0 ± 18.2a		58.7 ± 70.6a	84.7 ± 121.6a	
Ear tag	2	105.3 ± 22.4	17.0 ± 5.3b	91	105.7 ± 106.8	8.0 ± 15.7b	93	63.0 ± 75.9	4.0 ± 3.6ab	64	32.0 ± 25.5ab	6.3 ± 5.7b	93
Spray	2	52.3 ± 38.3	7.7 ± 9.9b	96	32.0 ± 28.7	6.0 ± 2.0b	95	13.7 ± 5.5	1.7 ± 0.6b	85	12.0 ± 9.5b	2.0 ± 2.6b	98
Untreated	3	61.3 ± 58.8	42.3 ± 4.9a		12.3 ± 14.7	3.7 ± 1.2		14.0 ± 10.8	4.0 ± 3.6a		45.0 ± 25.4a	12.3 ± 12.7a	
Ear tag	3	82.0 ± 40.6	16.3 ± 7.8b	61	7.0 ± 1.0	.3 ± 2.3	92	10.0 ± 1.7	7.0 ± 12.1a	0	12.5 ± 7.8b	1.7 ± 2.1b	86
Spray	3	120.0 ± 116.1	44.0 ± 40.6a	0	16.3 ± 23.1	2.0 ± 1.7	46	7.3 ± 7.6	0b	100	30.7 ± 31.8ab	8.3 ± 10.1ab	33
Untreated	4	114.0 ± 159.1	209.0 ± 269.3a		8.3 ± 4.0a	15.3 ± 16.3a		36.7 ± 34.2	11.0 ± 7.8a		26.7 ± 22.2	41.7 ± 48.3a	
Ear tag	4	66.3 ± 24.1	13.7 ± 8.6c	93	2.0 ± 2.7 b	0.7 ± 0.6b	95	15.3 ± 10.0	1.3 ± 1.2b	88	17.3 ± 7.1	4.3 ± 0.6b	90
Spray	4	136.3 ± 131.6	58.0 ± 38.9b	72	2.7 ± 1.2b	1.0 ± 0.0b	93	15.0 ± 9.6	2.3 ± 2.1b	79	36.7 ± 24.0	7.0 ± 4.4b	83
Untreated	5	39.0 ± 39.0	107.7 ± 36.6a		0.3 ± 0.6b	0.7 ± 0.6		6.3 ± 6.1a	1.7 ± 1.5		15.3 ± 10.1	14.7 ± 5.7a	
Ear tag	5	30.3 ± 11.7	12.0 ± 7.0b	89	2.7 ± 0.6a	1.0 ± 0.0	0	11.3 ± 4.0a	1.3 ± 1.5	24	25.3 ± 3.8	4.0 ± 2.0b	73
Spray	5	72.0 ± 36.9	88.0 ± 20.7a	18	0.7 ± 1.2ab	0.7 ± 0.6	0	2.0 ± 1.0b	0.7 ± 0.6	59	16.3 ± 11.1	13.3 ± 3.1a	10
Untreated	6	9.3± 5.5a	20.0 ± 5.0a		0	0		2.3 ± 2.1	2.3 ± 2.5		13.7 ± 9.6a	19.3 ± 13.0a	
Ear tag	6	6.0 ± 4.6 a	1.0 ± 1.0b	95	0	0	0	3.3 ± 1.2	0.7 ± 0.6	70	4.7 ± 0.6b	2.0 ± 1.7b	90
Spray	6	36.3 ± 23.1b	26.3 ± 15.6a	0	0.3 ± 0.6	0	0	3.7 ± 2.5	1.0 ± 1.0	57	14.3 ± 11.4a	2.0 ± 2.6b	90

[1]Week sampled

[2]Means in a column, separated by week sampled, and followed by a different letter grouping significantly differ (α=0.05); LSD (Proc MIXED, Littell et al. 2002)

[3]Percent feeding suppression by treatment

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THE EFFECT OF TICK SPECIES ON FECAL NEAR INFRARED SPECTROSCOPY PREDICTED DIET QUALITY OF CATTLE

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ABSTRACT: We conducted 2 trials with 12 cross-bred beef steers (250 ± 10kg) to determine the effect of cattle fever tick (CFT) species on fecal near infrared spectroscopy (FNIRS) predicted diet crude protein (CP) and digestible organic matter (DOM). Cattle experiencing an *Amblyomma americanum* tick burden have been reported to have lower FNIRS predicted diet quality during the peak tick blood feeding period (PEK) than the pre-tick blood feeding (PRE) period. The CFT species *Rhipicephalus (Boophilus) microplus* and *R. (B.) annulatus* have different life cycles and immunomodulation strategies than *A. americanum*. It is not known whether CFT will similarly affect fecal composition as detected via FNIRS. In both trials, animals were fed a 13 ± 1 % CP commercial mixed ration for 50 d. Fecal samples were collected daily and processed for FNIRS. Comparisons between PRE, PEK, and recovery (REC) periods of the tick blood feeding cycles were determined via analysis of variance procedures. In trial 1, 3 steers were infested (TRT) with *R. microplus* and 3 served as non tick-treated controls (CON). There were no overall differences ($P > 0.1$) in diet CP or DOM due to treatment. Within TRT, however, PRE DOM (64.68 ± 0.81). was less than ($P < 0.05$) PEK (68.06 ± 0.97). Period of the tick blood feeding cycle affected ($P < 0.05$) diet DOM in that PRE (64.94 ± 0.51) was less than PEK (66.86 ± 0.72). In trial 2, 3 steers were infested with *R. annulatus* and 3 served as non tick-treated controls. There were no differences ($P > 0.1$) in diet CP or DOM due to treatment. Period of the tick blood feeding cycle affected ($P < 0.05$) diet quality in that PRE CP (12.47 ± 0.33) was less than both PEK (13.74 ± 0.33) and REC (13.71 ± 0.39). Additionally, PRE DOM (65.42 ± 0.42) was less ($P < 0.05$) than REC (66.75 ± 0.38), but not PEK (66.45 ± 0.41). Within CON, CP was less ($P < 0.05$) for PRE (12.34 ± 0.60) than both PEK (14.23 ± 0.15) and REC (14.2 ± 0.30), and DOM was less ($P < 0.05$) for PRE (65.36 ± 0.85) than REC (67.32 ± 0.17), but not PEK (66.82 ± 0.15). *R. microplus* and *R. annulatus* differentially affected FNIRS predicted diet quality in growing pen-fed cattle. Knowledge of the effects of CFT on cattle diet quality could enhance fever tick surveillance in south Texas along the border with Mexico.

Key Words: cattle, cattle fever ticks, diet quality, fecal, near infrared spectroscopy

Introduction

Cattle diet quality, i.e. crude protein (CP) and digestible organic matter (DOM), has been predicted using near infrared spectroscopy of feces (FNIRS; Lyons and Stuth, 1992; Coates, 1998; Ksiksi et al., 2000; Boval et al., 2004). Discrimination between groups of cattle and horses with and without a tick burden has also been accomplished via FNIRS (Tolleson et al., 2007). Cattle experiencing a single blood feeding cohort of adult Lone Star (*Amblyomma americanum*) ticks have been reported to have lower FNIRS predicted diet quality during the peak tick blood feeding period than the pre-infestation period (Tolleson et al., 2002). Nutrition affects overall animal health, including immune function (Chandra, 1997). Cattle diet quality is lower in warmer/drier than in cooler/wetter climates (Craine et al., 2010). Tick species may be more or less prevalent in drought depending on species and geographic location (Hair et al.,1975; Wang et al., 2012). Lone Star ticks affected liver IGF-I mRNA (Tolleson et al., 2010), and plasma cortisol and IGF-I in growing beef steers (Tolleson et al., 2012), especially in those animals on a low plane of nutrition. Climate may thus interact not only with the ecology of tick species but also with host susceptibility to parasitism.

Ticks such as *Rhipicephalus (Boophilus) microplus* and *R. (B.) annulatus*, collectively known as cattle fever ticks (CFT) are known to carry the causative organisms (*Babesia bigemina* and *B. bovis*) for bovine babesiosis, or cattle tick fever (Pérez de León et al., 2010). Cattle tick fever was responsible for estimated direct and indirect costs to the cattle industry of \$130m yr^{-1} during the late 1800's and early 1900's (Graham and Hourrigan, 1977). *Rhipicephalus* species in the sub-genus *Boophilus* have different life cycles and immunomodulation strategies than *A. americanum* (Brake and Pérez de León, 2012). It is not known whether parasitism by these *Rhipicephalus* species will similarly affect cattle fecal composition as compared to *A. americanum*, and as detected via FNIRS. Although the U.S. has been free of CFT since their eradication in 1943, these ticks remain a threat to the livestock industry because they are established in Mexico (Miller et al., 2013). A permanent quarantine zone in south Texas along the border with Mexico buffers CFT incursions into the U.S. (Pérez de León et al., 2012). Knowledge of the effects of CFT on predicted diet quality could be applied to develop detection

methods to enhance surveillance and bioforensic applications in the permanent quarantine zone managed by the Cattle Fever Tick Eradication Program. We conducted 2 feeding trials with growing cross-bred beef cattle infested with CFT to determine their effect on FNIRS predicted diet quality.

Materials and Methods

All animal procedures were conducted at the USDA-ARS Cattle Fever Tick Research Laboratory in Edinburg, Texas in accordance with protocols approved by the Institutional Animal Care and Use Committee. All steers (250 ± 10kg) were maintained in outdoor environments in an open barn with concrete-floored pens and fed an ~13% CP commercial mixed ration (Table 1) for ~50 d. Trial 1 was conducted with *R. microplus* and Trial 2 utilized *R. annulatus*. In both trials, 3 animals were each infested with 5,000 larvae (TRT) and 3 animals served as non tick-treated controls (CON). Tick feeding cycle for both CFT species was 21 days. Ticks were raised under standard laboratory conditions as described by Davey et al. (1980). Larvae were placed on the animals at experimental d 4 and counted on d 20 to 30 to define PRE (d 0), PEK (d 22 Trial 1; d 23 Trial 2) and REC (d 42 trial 1; d 48 Trial 2) periods.

Fecal samples were collected for each animal from the floor of the pens daily and processed for FNIRS as per the method of Lyons and Stuth (1992). Briefly, each fecal sample was dried overnight at 60^o C, ground to 1mm particle size in a laboratory mill and then re-dried at 60^o C prior to scanning in quartz lens cups. Infrared spectra (1100 to 2500nm) were obtained using a Foss NIRS® 6500 spectrometer. Diet quality (% CP, % DOM) calibrations (R^2 = 0.95, SE cross validation = 0.91; and R^2 = 0.91, SE cross validation = 1.59 respectively) were developed prior to this experiment using multivariate regression procedures (Johnson, 1998) on paired diet chemistry/fecal spectra calibration sets (n = 953 and 794 respectively).

Minimum and maximum temperature and % relative humidity were obtained from the US Animal Performance Weather Data System (http://cnrit.tamu.edu/cgi-bin/nutbalweather). Within trial, differences between FNIRS predicted diet quality due to tick treatment and period of the respective tick species blood feeding cycles were determined via analysis of variance procedures (Steel and Torrie, 1980). Mean separation was accomplished by Fisher's least-significant-difference test (Steel and Torrie, 1980).

Results

Mean and standard error values for each treatment group and period in both trials are presented in Table 2. There were no overall differences (P > 0.1) in FNIRS predicted diet CP or DOM due to treatment during Trial 1. Within TRT, however, PRE DOM (64.68 ± 0.81) was less than (P < 0.05) PEK (68.06 ± 0.97) but not REC (65.70 ± 0.39). Also within TRT, CP was numerically less (P = 0.14) for PRE (12.69 ± 0.46) than PEK (14.24 ± 0.45) and REC (13.42 ± 0.48). Period of the tick blood feeding cycle affected (P < 0.05) FNIRS predicted diet DOM in that PRE (64.94 ± 0.51) was less than PEK (66.86 ± 0.72) but not REC (65.83 ± 0.60). Average daily maximum and minimum temperatures were 36.7 ± 0.2 and 24.7 ± 0.5^o C respectively. Average daily % relative humidity was 81.2 ± 0.9.

There were no differences (P > 0.1) in FNIRS predicted diet CP or DOM due to treatment during Trial 2. Period of the tick blood feeding cycle affected (P < 0.05) FNIRS predicted diet quality in that PRE CP (12.47 ± 0.33) was less than both PEK (13.74 ± 0.33) and REC (13.71 ± 0.39). Additionally, PRE DOM (65.42 ± 0.42) was less (P < 0.05) than REC (66.75 ± 0.38) but not PEK (66.45 ± 0.41). Within CON, CP was less (P < 0.05) for PRE (12.34 ± 0.60) than both PEK (14.23 ± 0.15 and REC (14.2 ± 0.30), and DOM was less (P < 0.05) for PRE (65.36 ± 0.85) than REC (67.32 ± 0.17) but not PEK (66.82 ± 0.15). Average daily maximum and minimum temperatures were 32.7 ± 1.7 and 19.6 ± 1.6^o C respectively. Average daily % relative humidity was 78.2 ± 1.3.

Discussion

Our preliminary findings suggest that *R. microplus and R. annulatus* ticks differentially affected FNIRS predicted diet quality during PRE, PEK, and REC in growing pen-fed cattle. To be clear, this does not imply that actual diet quality was different between the two groups. Feed offered was consistent across trials and treatments. Rather, our results indicate that there were measureable differences in fecal chemistry as evidenced by predicted diet quality. In general, FNIRS predicted diet quality of *R. microplus*-treated cattle appeared to increase during the tick blood feeding cycle (Figure 1) as compared to no apparent change for cattle infested with *R. (B.) annulatus* (Figure 2). These results are in contrast to our previous observation (Tolleson et al., 2002) of a reduction in both FNIRS predicted diet CP and DOM during peak blood feeding periods in growing beef cattle infested with *A. americanum*. Several possibilities exist for these observed differences in FNIRS predicted diet quality.

First, there is a difference in temporal and fluid dynamics of blood feeding resulting in different host-stress dynamics. *Amblyomma americanum* is a 3-host tick and prior studies focused on blood feeding of adult ticks over a 10 to 14 day feeding period (Tolleson et al. 2002). Converesely, CFT complete their life cycles on 1 host with blood feeding progressively accomplished by larvae, nymphs, and adults in 20 to 30 days (Tatchell, 1967).

Second, different immunomodulation strategies may produce different host responses. Variation in salivary gland function and products exists across ixodid tick genera, and perhaps species (Sauer et al., 2004). These secretions contain pharmacologically active proteins and lipids used in feeding lesion development and continuous modulation of inflammatory, cellular, and humoral immune system responses (Jaworsky, 2003; Brake and Pérez de León, 2012). Metabolic and endocrine indicators such as IGF-I or cortisol can be affected by *A. americanum* infestations in cattle (Tolleson et al., 2010 and 2012). It is not known what effects CFT might have on such indicators.

Third, environmental conditions varied between the studies. Trial 1 was conducted in the summer under

warmer and more consistent temperatures. Trial 2 immediately followed in the autumn with cooler but more variable temperatures. Relative humidity was similar between the two trials. Our previous work with *A. americanum* was conducted indoors under controlled conditions (21.0 ± 1.0° C, ~50% relative humidity). Temperature and humidity affect animal ingestive behavior (Mateus et al., 1992), passage rate (Bernabucci et al., 1999), and nutritional requirements (NRC 2000). Each of these could affect predicted diet quality via changes in fecal chemistry.

Implications

Fecal NIRS can be used to monitor diet quality of range beef cattle in the southwestern US (Lyons, 2010; Tolleson and Schafer, 2010). Knowledge of the effects of tick parasitism on FNIRS predicted cattle diet quality could be used to inform the development of technologies applied to enhance CFT surveillance and bioforensic applications in the permanent quarantine zone managed by the Cattle Fever Tick Eradication Program. Further research is warranted to determine if this methodology can distinguish between cattle infestation with native or invasive tick species that threaten the livestock industry in the U.S.

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Table 1. Composition of total mixed ration fed to growing beef steers during tick infestation trials.

Ingredients	% As Fed
Grain by-products	47.5
Grain products	24.8
Roughage products	20.6
Forage products	3.5
Plant protein products	1.3
$CaCO^3$, Salt, Trace mineral	2.3
Total	100

Table 2. Mean and standard error diet crude protein and digestible organic matter in growing beef steers during three periods of the tick blood feeding cycle as predicted by fecal near infrared spectroscopy.

	Trial 1. *Rhipicephalus microplus*			
	Crude protein (%)		Digestible Organic Matter (%)	
Period	Tick-treated	Control	Tick-treated	Control
Pre-infestation (d 0)	12.69 ± 0.46	13.13 ± 0.42	64.68 ± 0.81	65.20 ± 0.75
Peak Stress (d 22)	14.24 ± 0.45	13.01 ± 0.06	68.07 ± 0.97	65.65 ± 0.48
Recovery (d 42)	13.42 ± 0.48	13.37 ± 0.71	65.70 ± 0.39	65.96 ± 1.27

	Trial 2. *Rhipicephalus annulatus*			
	Crude protein (%)		Digestible Organic Matter (%)	
Period	Tick-treated	Control	Tick-treated	Control
Pre-infestation (d 0)	12.60 ± 0.39	12.34 ± 0.60	65.48 ± 0.37	65.36 ± 0.85
Peak Stress (d 23)	13.24 ± 0.54	14.22 ± 0.16	66.08 ± 0.82	66.81 ± 0.15
Recovery (d 48)	13.21 ± 0.65	14.20 ± 0.30	66.18 ± 0.61	67.32 ± 0.17

Figure 1. The effect of period during the tick blood feeding cycle on fecal near infrared spectroscopy predicted diet crude protein and digestible organic matter in growing beef steers. Trial 1 (*Rhipicephalus microplus*).

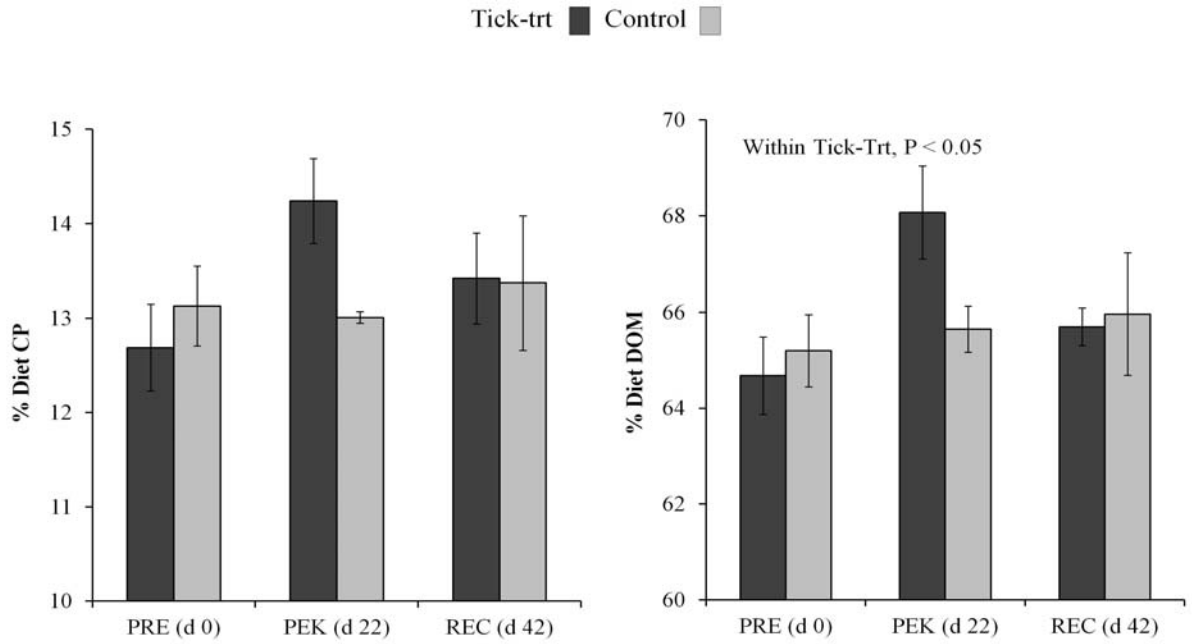
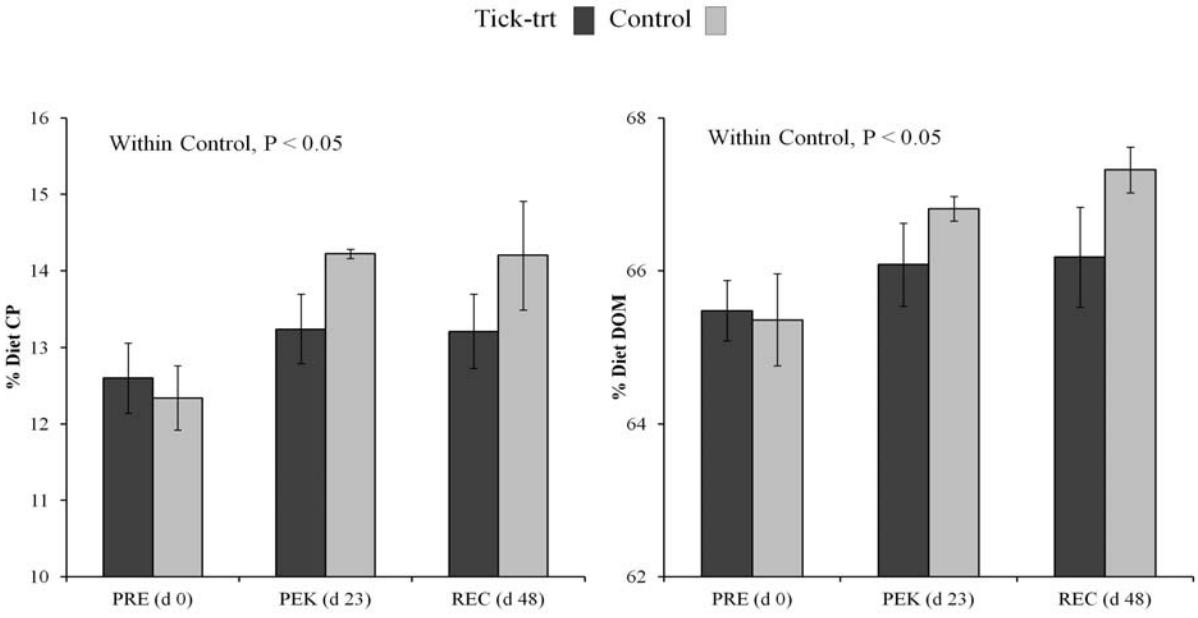


Figure 2. The effect of period during the tick blood feeding cycle on fecal near infrared spectroscopy predicted diet crude protein and digestible organic matter in growing beef steers. Trial 2 (*Rhipicephalus annulatus*).



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IMPACT OF NUMBER OF ESTROUS CYCLES EXHIBITED PRIOR TO START OF BREEDING ON REPRODUCTIVE PERFORMANCE IN BEEF HEIFERS

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ABSTRACT: Objective of this research was to evaluate effect of number of estrous cycles exhibited prior to breeding on reproductive performance of replacement beef heifers. A total of 1,176 composite heifers (½ Red Angus, ¼ Charolais, ¼ Tarentaise) were evaluated over a 9-yr period. Circulating concentrations of progesterone measured in blood samples collected at 9- to 11- d intervals, beginning at an average age of 331 d, were used to estimate date of first estrus (estrus = 6 d prior to date of first sample ≥ 1 ng/mL). Heifers were categorized into one of five groups by number of estrous cycles exhibited (21-d intervals) before start of breeding; 0 (non-pubertal; n = 395), 1 (n = 205), 2 (n = 211), 3 (n = 116), or > 3 (n = 249). Mean BW and age at start of breeding differed ($P < 0.001$) by categorization; non-pubertal heifers were 5 d younger (420 ± 1 d old) and 15 kg less BW (304 ± 2 kg) compared to other groups. Pregnancy rates differed ($P = 0.007$) by category; heifers that were non-pubertal before start of breeding had lower pregnancy rates (84 ± 2%; $P < 0.05$) than heifers exhibiting 1 (90 ± 2%) or > 3 (94 ± 2 %) estrous cycles before start of breeding, but only tended to differ ($P < 0.15$) from the 2 (88 ± 2%) and 3 (89 ± 3%) estrous cycle groups. Heifers that were non-pubertal at start of breeding had 3- to 5-d longer ($P < 0.05$) interval (300 ± 1 d) from start of breeding to calving than other groups. Rebreeding pregnancy rates of 2-yr old cows (n = 781) were lowest ($P < 0.05$) for heifers from the 0 estrous cycle group (62 ± 3%), followed by 1 (71 ± 4%) and 2 (73 ± 4 %) estrous cycle categories, which were less ($P < 0.05$) than 3 (83 ± 5%) and > 3 (86 ± 4%) estrous cycle categories. Attainment of puberty before start of breeding was associated with increased heifer pregnancy rate, with little or no benefit from having more than one estrous cycle prior to start of breeding. In contrast, second season pregnancy rate was positively associated with number of estrous cycles expressed prior to start of first breeding season.

Key words: beef heifers, puberty, reproduction

Introduction

Obtaining high pregnancy rates in replacement females is a common goal among beef cattle producers. One industry guideline recommends heifers should be managed so they achieve puberty in sufficient time to experience multiple estrous cycles before start of breeding. This recommendation is based on research that demonstrated a 21 percentage point increase in pregnancy rate in heifers inseminated on their third estrus compared to heifers inseminated on their first estrus (Byerley et al., 1987). It was concluded from these results that fertility of the first pubertal estrus was inferior to the third estrus. Additional studies confirming this conclusion are lacking. It is expected that there has been substantial genetic change in the cattle population since these data were collected over 25 yr ago (Endecott et al., 2013). Furthermore, Byerley et al. (1987) had potentially confounding effects of heifer age and BW at time of breeding between treatment groups. Heifers inseminated at first estrus were 53 d younger (322 vs. 375 d) and 31 kg lighter (295 vs. 326 kg) than heifers inseminated on their third estrus. Objective of this research was to provide a more thorough evaluation of whether number of estrous cycles exhibited before start of breeding affects pregnancy rates of replacement beef heifers.

Materials and Methods

All research protocols used in this study were approved by the USDA, ARS, Fort Keogh Livestock and Range Research Institutional Animal Care and Use Committee. Cows used in this study were a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise). Females studied (n = 1,176) represent a randomly selected population produced (Julian date of birth 96 ± 15) over a 9-yr period by mating CGC dams and sires with consideration given to minimize inbreeding, but without emphasis on production traits. At weaning, heifers were placed in a feedlot and subsequently assigned to either control or restricted feeding levels for a 140-d period (Roberts et al., 2009b). Heifers averaged 245 ± 19 d of age and 220 ± 25 kg BW at initiation of the feeding period. Control heifers were fed to appetite and restricted heifers were fed at 80% of that consumed by controls adjusted to a common BW basis (BW measurement and feed adjustments made at 28-d intervals). The 140-d period ended 40 ± 11 d before start of breeding. Heifers were then managed the same throughout the breeding season. A prebreeding BW was taken 0 to 29 d (varied over years) before start of breeding. This population provided an opportunity to evaluate the objective using heifers developed at different rates of growth during the postweaning period.

Circulating concentrations of progesterone were used to estimate date of first estrus. Beginning at approximately 331 d of age through the start of breeding, 3 to 9 mL of blood were collected from each heifer by coccygeal venipuncture at 9- to 11-d intervals. After collection, blood was placed on ice and stored overnight at 4° C. Blood was then centrifuged at 1,200 × g for 30 min. Serum was harvested and stored at -20° C for subsequent analysis of progesterone. Concentrations of progesterone in serum 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). Intra- and inter-assay CV were 8 and 16%, respectively, and assay sensitivity was 0.08 ng/mL. For the purpose of this study, date of first estrus was assumed to occur 6 d before date of first progesterone sample ≥ 1 ng/mL. Heifers were classified into 1 of 5 categories based on the estimated number of estrous cycles exhibited before start of breeding, assuming a 21-d interval for each estrous cycle: category = 0 if no progesterone sample was ≥ 1 ng/mL (non-pubertal; n = 395); category = 1 if number of d between first progesterone sample ≥ 1 ng/mL and start of breeding was < 15 d (n = 205); category = 2 if number of d between first progesterone sample ≥ 1 ng/mL and start of breeding was 15 to 35 d (n = 211); category = 3 if number of d between first progesterone sample ≥ 1 ng/mL and start of breeding was 36 to 56 d (n = 116); category > 3 if number of d between first progesterone sample ≥ 1 ng/mL and start of breeding was > 56 d (n = 249).

Beginning approximately June 1 each year, at an average age of 425 ± 15 d (youngest and oldest heifer over the 9 yr was 370 and 465 d, respectively), heifers were bred by AI followed by natural mating for the duration of a 48- to 55-d breeding season (n = 5 yr) or a 46- or 62-d breeding season with natural mating only (n = 4 yr). In the 5 yr heifers were subjected to AI, estrous synchronization was used to facilitate AI. Estrous synchronization protocols varied across yr with a CO-synch + controlled internal drug releasing devise (**CIDR**; Pfizer Animal Health, New York, NY; Lamb et al., 2006) protocol used in 2 yr and protocols with either 1 or 2 injection of $PGF_{2\alpha}$ used in 3 yr. Depending on the breeding protocol used, duration of breeding season was adjusted to provide heifers 3 opportunities to be inseminated if pubertal at start of breeding.

In December of each year, heifers were divided into their postweaning treatment groups to allow for different levels of supplemental feeding through the winter as previously described (Roberts et al., 2009a). For the majority of the study, pasture forage was readily available for winter grazing and the only additional supplemental protein provided was alfalfa cubes or hay, depending on yr. Supplement was fed either daily or every other day to achieve 1.8 kg/d or 1 kg/d offered for each control or restricted heifer, respectively. Days when access to pasture was limited due to snow cover, heifers were provided 10.9 or 9.1 kg alfalfa hay/d for control and restricted treatments, respectively. At approximately 2- to 4-wk before start of calving, heifers were recombined. Date of calving and interval from start of breeding to calving was recorded for each animal. Pregnancy rates for the second breeding season were recorded for all cows nursing a calf (n = 781; data not available for last yr of the study).

Statistical analysis. Data were analyzed with SAS (SAS Inst. Inc., Cary, NC). Differences in age and BW of heifers classified into the different estrous cycle categories were evaluated by the GLM procedure with a model that included effects of yr (n = 9), postweaning feeding treatment (n = 2), estrous cycle category (n = 5), and interaction of feeding treatment and estrous cycle category. The same model was used to evaluate effect of estrous cycle category on interval between start of breeding and calving. Interaction of feeding treatment and estrous cycle category was not significant ($P > 0.22$) for any variable, and was thus removed from the model. Effects of estrous cycle category on pregnancy rate as a heifer and a 2-yr old cow (rebreeding pregnancy rate; data not available for last yr of study) were evaluated using Logistic regression with yr, feeding treatment and estrous cycle category as fixed effects. Year accounted for significant variation in all analysis ($P < 0.001$). However, these results are not presented for sake of brevity. Effect of feeding treatment is discussed when $P < 0.05$. Values presented represent least square means and SE.

Results and Discussion

Because age and BW influence onset of puberty, comparison of these factors were made across the estrous cycle categories. Heifers that were non-pubertal at start of breeding weighed less ($P < 0.001$) before start of breeding and were younger at start of breeding ($P \leq 0.001$) compared to heifers that exhibited 1 or more cycles before start of breeding (Table 1). Heifers that exhibited 1 or 2 estrous cycles before start of breeding tended to be lighter ($P \leq 0.12$) than heifers exhibiting 3 estrous cycles before start of breeding, and were lighter ($P < 0.02$) than those exhibiting > 3 estrous cycles before start of breeding. Body weight before start of breeding was similar between heifers exhibiting 3 and > 3 estrous cycles ($P = 0.64$, Table 1). Heifers developed on restricted level of feeding had lower BW (311 ± 1.4 kg; $P < 0.001$) before start of breeding than the control group (327 ± 1.4). Age of heifers at start of breeding was similar for heifers that exhibited either 1, 2 or 3 estrous cycles before start of breeding (426 d of age), and these 3 groups were younger ($P < 0.01$) than heifers exhibiting > 3 estrous cycles before start of breeding (430 d, Table 1). These results are consistent with other literature demonstrating association of BW and age with onset of puberty (reviewed in Patterson et al., 1992; Funston et al., 2012).

Pregnancy rates differed ($P = 0.007$) by number of estrous cycles exhibited before start of breeding (Table 1). Heifers that were non-pubertal before start of breeding had lower pregnancy rates (84 ± 2%; $P < 0.05$) than heifers exhibiting 1 (90 ± 2%) or > 3 (94 ± 2 %) estrous cycles before start of breeding, but only tended ($P < 0.15$) to differ from heifers that exhibited 2 (88 ± 2%) or 3 (89 ± 3%) estrous cycles before start of breeding. Interval from start of breeding to calving was influenced by estrous cycle classification (Table 1); heifers that were non-pubertal at start of breeding had a 3- to 5-d longer ($P < 0.05$) average interval than heifers that exhibited 1 or more estrous cycles before start of breeding. The longer interval between start of breeding and calving is indicative of a later conception date for heifers that were non-pubertal at beginning of the breeding season. The impact of pubertal status at initiation of breeding on pregnancy rate is in agreement with others (Patterson et al., 1992).

In the present study, heifers that had not exhibited estrus before start of breeding would have their first opportunity to conceive on their pubertal estrus. Heifers expressing 2 estrous cycles before start of breeding would have their first opportunity to conceive on their third estrus. These groups of heifers are comparable to the treatment

groups of Byerley et al. (1987). While both studies provide evidence for lower pregnancy rates at first estrus compared to third estrus, magnitude of difference was much greater in the study by Byerley et al. (1987) than in the present study (20 vs. 5 percentage point reduction). In addition, results from the present study do not support the theory that fertility is improved in heifers expressing more than one estrous cycle before start of breeding. The discrepancy in the results obtained by Byerley et al. (1987) and the present study is likely due to differences in experimental design and may also reflect genetic change in heifers over time, as suggested in the reviews by Funston et al. (2012b) and Endecott et al. (2013). In the study by Byerley et al. (1987), insemination date was determined by when heifers expressed estrus, whereas initiation of breeding remained constant in the present study. Heifers inseminated at first estrus were approximately 11 mo old at time of breeding in the study by Byerley et al. (1987), whereas breeding was not initiated until approximately 14 mo of age in the present study, which is more representative of current industry practices. The importance of differences in age of breeding between these 2 studies was alluded to by Byerley et al. (1987) where increased age resulted in increased pregnancy rates for heifers inseminated at first estrus, but not in heifers inseminated on their third estrus. Another difference between the 2 studies is heifers were only provided one opportunity to conceive in Byerley et al. (1987), whereas, multiple opportunities for conception were possible in the present study.

Second season pregnancy rates were influenced ($P < 0.019$) by estrous cycle category before the first breeding season (Table 1). Lowest ($P < 0.05$) pregnancy rates occurred in the 0 estrous cycle group followed by 1 and 2 estrous cycle categories, which were less ($P < 0.05$) than 3 and > 3 estrous cycle categories. Second season pregnancy rate was also influenced ($P = 0.04$) by dietary treatment during the postweaning period and winter supplementation, with heifers developed on restricted feeding having lower pregnancy rates (72%) than control-fed heifers (78%). The mechanism by which estrous cycle category at first breeding affects second season pregnancy rates remains to be established. Further elucidation of biological mechanisms may result in strategies to optimize second season pregnancy rates. The impact of nutritional treatment on pregnancy rate demonstrates reproduction can be improved with greater feed inputs. However, it is not clear if the improved pregnancy rates are achieved by altering timing of puberty through management strategies in the postweaning period, by providing additional nutrition during winter, or the combination of both. It is expected that at least some variation among heifers across estrous cycle categories is heritable (i.e., early vs. late sexual maturity). If this is significant, then altering management to promote earlier puberty may improve heifer pregnancy rate, but actually be counter-productive over the long-term by masking genetic differences contributing to improved retention over time.

Differences due to age may also have significant effects on rebreeding performance. Impact of age appears to be a function of when the animal was born (i.e., early vs. later in the calving season) and also when an animal conceives (those conceiving later may be older at calving). In the present study, heifers in the >3 estrous cycle group were older at start of breeding than other heifers, and this group also had one of the highest second season pregnancy rate. In contrast, heifers that have longer intervals from start of breeding to calving could potentially be older at calving. Rogers et al., (2004) observed a trend that cows ≤730 d old at first calving were at less risk of being culled than cows >730 d of age at first calving. This observation is consistent with the recommendation heifers be bred to calve early to provide more time for resumption of estrus to occur before start of subsequent breeding.

Implications

Attainment of puberty before start of breeding was associated with a modest increase in pregnancy rate, with little or no benefit from having more than one estrous cycle expressed before start of breeding. Thus, these results do not support the recommendation to manage heifers to allow 2 or more estrous cycles before start of breeding. In contrast, second season pregnancy rate was positively associated with number of estrous cycles expressed before start of first breeding season. These findings support the need for additional research to substantiate the potential impacts that timing of puberty before breeding may have on subsequent pregnancy rates the next year and investigate potential intervention strategies.

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Table 1. Weight, age, and reproductive performance of heifers classified based on number of estrous cycles exhibited before start of breeding

	Number of estrous cycles before start of breeding						
	0	1	2	3	>3	SE[1]	*P*-value[2]
Heifers first season, n	395	205	211	116	249		
BW before start of breeding, kg	305[a]	319[b]	319[b]	325[b,c]	325[c]	2.9	< 0.001
Age at start of breeding, d	420[a]	426[b]	426[b]	426[b]	430[c]	1.4	< 0.001
Heifer pregnancy rate, %	84[a]	90[b]	88[a]	89[ab]	94[b]	2.9	< 0.008
Start of breeding to calving, d	300[a]	297[b]	295[b]	295[b]	296[b]	1.4	< 0.001
Two-yr-old cows, second season, n	228	144	132	87	190		
Second season pregnancy rate, %	62[a]	71[b]	73[b]	83[c]	86[c]	4.7	< 0.019

[1] Largest SE.

[2]P-value for effect of estrous cycle category.

[a-c] Means without a common superscript differ ($P \leq 0.05$).

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A PRELIMINARY ASSESSMENT OF YEAR LONG RELATIVE LOOSE MINERAL INTAKE AND RANGE COW PRODUCTIVITY IN NORTHERN GREAT PLAINS

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ABSTRACT: Assessment of the effectiveness of supplementary mineral nutrition in range cattle to promote important economic traits is lacking due a paucity of methods to measure cause and effect relationships, dynamic nature of diet mineral concentrations, shifting requirements and a lack of mineral intake quantification. This study evaluated relative mineral intake and its association with calf BW at birth and weaning, cow BW change and calving interval (d). Cross-bred cows grazed native range with access to mineral tubs containing 34% salt, 57% macro/microminerals, 9% distillers grains and 1% titanium (Ti) from August 2010 to November 2011. Rectal fecal samples were collected at 1 or 2 mo intervals and were analyzed for Ti content. It was assumed that fecal Ti was positively related to mineral consumption. Cows were assigned to one of four fecal Ti groups based on their mean Ti concentration: (1) low (3 to 5 ppm, n = 23), (2) mid-low (5 to 6 ppm, n = 36), (3) mid-high (6 to 7 ppm, n = 26) and (4) high (7 to 11 ppm, n = 21). Data were analyzed using Proc Mixed (SAS) and a model including Ti groups and cow age (2 to 11 years) as a covariate. Average consumption based on amount of mineral fed was greatest ($P<0.01$) during forage dormancy and spring growth (53 g hd-1 d-1) and lowest during late growing season (38 g hd-1 d-1). Regardless of fecal Ti , all groups had similar 2011 calf BW at birth and weaning, cow BW change (2010-2011) and 2011 to 2012 calving interval (35.9, 37.3, 38.6 and 35 ± 1.4 kg, $P < 0.22$; 232.7, 230.9, 226.4 and 230 ± 6.4 kg, $P = 0.89$; 14.5, 8.1, 0.5, 1.8 ± 9.5 kg, $P = 0.71$; and 365, 356, 366 and 365 ± 5 d, $P = 0.85$; respectively for low, mid-low, mid-high and high). In addition, mean fecal Ti in 18 non-pregnant and 88 pregnant cows were 6.22 ppm and 5.95 ppm ($P = 0.68$), indicating no differences in mineral consumption. Range in mean fecal Ti of individual cows represented a 3-fold divergence in Ti dilution indicating a magnitude difference in mineral consumption. If mineral consumption was a primary production limitation in the year of this study, then greater differences would be expected for the production traits evaluated.

Key words: mineral, range cow, supplement

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Introduction

Factors influencing voluntary loose mineral consumption have been subject to speculation. Rangeland and range cattle managers expect supplementation of deficient minerals to improve forage utilization and animal production. However, few studies have assessed mineral consumption and range cow productivity in an extensive grazing setting (Underwood and Suttle. 1999). Aspects of the environment including season of the year, water salinity, daily temperature, salt bush frequency, forage maturity, vegetation DM content and others have been suggested as important contributors to variability in mineral intake. Few studies have investigated the relationships between mineral intake, environmental changes that occur over a year and cow-calf responses. Understanding of intake variability should allow for techniques to be developed to insure closer realization of the targeted consumption and how it relates to animal performance. This study was designed to evaluate variation in herd mineral intake and the relationship of that variation to cow and calf BW change and reproductive success.

Materials and Methods

All research protocols used in this study were approved by Animal Care and Use Committee. This study was conducted at the USDA-ARS Fort Keogh Livestock and Range Research Laboratory near Miles City, MT from August 2010 through November 2011. Native vegetation on the 22,500-ha research station consists of a grama-needlegrass-wheatgrass (Bouteloua-Stipa-Agropyron) mix. The long-term average precipitation is 343 mm with about 60-70 % occurring during the mid-April through mid-September growing season. One hundred and six cross-bred cows grazed native range with access to 2 rubber mineral tubs containing 34% salt, 57% inorganic macro and microminerals, 9% distillers grains (as an attractant) and 1% titanium dioxide (Ti) from August 2010 to November 2011 (calcium, 11.5%, phosphorus, 8%, magnesium, 4%, potassium 2%, copper 2000 ppm, zinc 2000 ppm, manganese 0 ppm, selenium 13 ppm, and vitamin A, units/lb. 120,000). Target intake was 58g hd^{-1} d^{-1}. titanium dioxide was added to the mix to serve as a fecal marker to be associated with mineral mix consumption. Salt and mineral presented to livestock in rubber tubs was available for consumption continuously. Quantities of salt and mineral were recorded at the time of addition to tub.

Rectal fecal samples were collected at 1 or 2 mo intervals and were analyzed for Ti content. It was assumed that mean concentration of fecal Ti was positively associated with mineral consumption. Within 4 h after collection fecal samples were placed in a forced air oven and dried at 60^0C in a forced air oven. Samples were ground through a 1 mm screen using a Wiley mill. Two grams of each fecal sample was weighed into 50 mL tubes. Ten mL of 70% nitric acid was added to each tube. Tubes sat overnight under a laboratory hood and then placed in a heat block at 95^0C for 7 h. Tubes were monitored to keep 10 mL of nitric acid inside at all times. Tubes sat overnight in hood to cool. Samples were diluted with 40 mL of MQ water. Each sample was mixed and filtered using a 0.45 micron filter with vacuum. The filtered sample was transferred to 15ml falcon tubes for analysis using inductively-coupled plasma mass spectrometry analytical technique (USFS Bozeman Fish Technology Center, Bozeman, MT).

Mean Ti concentrations of fecal samples (maximum 11) for each cow were used to assign animals to one of four fecal Ti groups: (1) low (3 to 5 ppm, n = 23), (2) mid-low (5 to 6 ppm, n = 36), (3) mid-high (6 to 7 ppm, n = 26) and (4) high (7 to 11 ppm, n = 21) (Figure 2). Various production measurements were recorded during the 16 mo experiment. These measurements included; calf BW at birth in 2010 - 2012, calf BW at weaning in 2010 - 2011, cow BW at weaning in 2010 - 2011, cow BW change (Aug 2010 to Nov 2011), calving interval from 2010-2011 and calving interval from 2011-2012.

Statistical Analysis

Mineral and production data were analyzed as a completely randomized arrangement of treatments using the MIXED procedure of SAS (SAS Institute, Cary, NC) with cow as the experimental unit. The model to analyze production responses included Ti groups and cow age (2 to 11 years) as a covariate. The model for mineral intake included 3 vegetative phenological seasons. Significance was determined at $P \leq 0.05$.

Results and Discussion

Average consumption was less than the target amount of 58 g hd^{-1} d^{-1}. Based on amount of mineral fed the greatest consumption occurred ($P < 0.01$) during forage dormancy (winter) and spring growth (53 g hd^{-1} d^{-1}) and lowest during summer late growing season (38 g hd^{-1} d^{-1}) (Figure 1.). Amount of mineral consumption in the late growing season was likely inflated due to potential mineral consumption of calves, which were observed loafing around mineral tubs. Our methods did not allow for differential measurement of mineral disappearance attributed to calves or cows. The range in mean fecal Ti attributed to mineral consumption by individual cows represented a 3-fold difference in Ti dilution likely indicating large variation in mineral consumption (Figure 2.).

In spite of the large range in fecal Ti concentrations, all fecal Ti concentration groups had similar ($P > 0.22$) production responses for calf BW at birth and weaning in 2011, cow BW at weaning (2010 and 2011), cow BW change (2010-2011) and calving interval from 2011 to 2012 (Table 1). In addition, mean fecal Ti concentrations in 18 non-pregnant and 88 pregnant cows were 6.22 ppm and 5.95 ppm ($P = 0.68$), indicating no differences in mineral consumption, which suggests reproductive success was not driven by mineral intake. Suttle (2000) suggested the surest diagnosis of health or performance limiting deficits in mineral supply is provided by responses to specific additions to supply. The mineral provided to the cows in this study was specifically formulated to address forage mineral deficiencies (NRC 2000). Since we found no differential productivity measures in cows or their calves related to the assumed amount of mineral supplement consumed we can conclude that the amount of mineral consumed was not an important determinant of productivity. However, during the months that this study was conducted it is possible that the yearly variation (i.e. high precipitation) may have masked responses to mineral that may occur in normal or less than normal rainfall years.

In contrast to our findings, Mundell et al. (2012) have reported different responses in AI pregnancy percentages due to the form of mineral fed; however, reported no differences in cow BW and BCS from initiation of the study to calving and from AI to weaning nor calf BW at birth, ADG, and age-adjusted weaning BW. Manspeaker et. al. (1987) reported improved reproductive performance in dairy cows supplemented with organic minerals over cows given no mineral supplementation. Stanton et al. (2000) utilized 300 Angus cows in a 209 d trial that was initiated prior to calving where cows were supplemented with either a low level inorganic, a high level of inorganic, or a high level organic mineral supplement. Cows on the high level of inorganic minerals lost most BW among the mineral treatments. Cattle in the organic mineral treatment exhibited better pregnancy rates to AI and increased calf ADG; however, overall pregnancy rates did not differ. Overall, production responses to mineral intake are variable and maybe due to environmental conditions during the year the studies were conducted.

Implications

Herd mineral consumption during the winter and spring was 12% less and in summer 37% less than the target amount. However, individual intake as shown by the range in fecal Ti was nearly 3-fold different suggesting large animal to animal variation with some animals consuming in excess while others were short of target amount. If mineral in fact limited cow productivity we would have predicted lower productivity in those cows that were found to have the lowest concentration of marker in their feces. However, this study indicates that the relationship with fecal Ti and productivity was not consistent and demonstrated that in the year this study was conducted the least amount of mineral consumed was sufficient to meet the cows' requirements.

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Table 1. Weight, age, and reproductive performance of cows and calves consuming a self fed salt-mineral mix

	Fecal Titanium Treatment Group					
	low	Mid-low	Mid-high	High	SE[1]	*P*-value[2]
Calf Birth Weight 2010, kg	36.1	38.1	34.1	36.9	2.6	0.06
Calf Birth Weight 2011, kg	36.7	38.1	38.2	33.9	2.8	0.09
Calf Birth Weight 2012, kg	39.0	36.0	37.1	34.0	3.0	0.14
Calf Weaning Weight 2010, kg	243.7	241.2	233.6	229.2	16.4	0.56
Calf Weaning Weight 2011, kg	241.5	236.5	221.5	218.2	14.8	0.06
Cow Weaning Weight 2010, kg	541[a]	547.0[a]	516.8[ab]	486.6[b]	29.0	0.01
Cow Weaning Weight 2011, kg	551.5[a]	547.4[a]	515[ab]	493.9	29.6	0.01
Cow Body Weight Change, kg	7.8	2.5	5.8	13.4	20.2	0.86
Calving Interval 2010-2011, d	365.78	362.6	362.9	368.7	3.4	0.66
Calving Interval 2011-2012, d	363.17	354.1	367.4	370.8	10.2	0.62

[1] Largest SE.

[2]P-value for effect

[a-c] Means without a common superscript differ ($P \leq 0.05$).

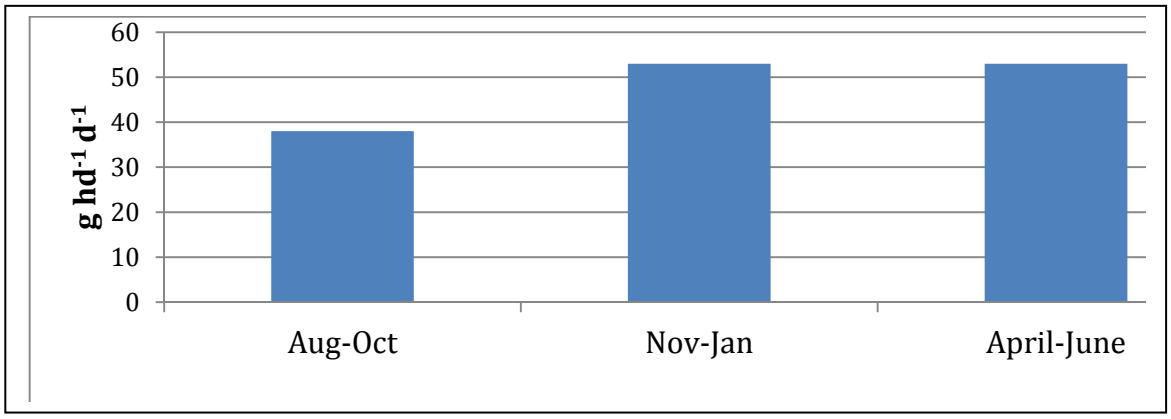

Figure 1. Average mineral daily consumption daily by growing season (P<0.01) .

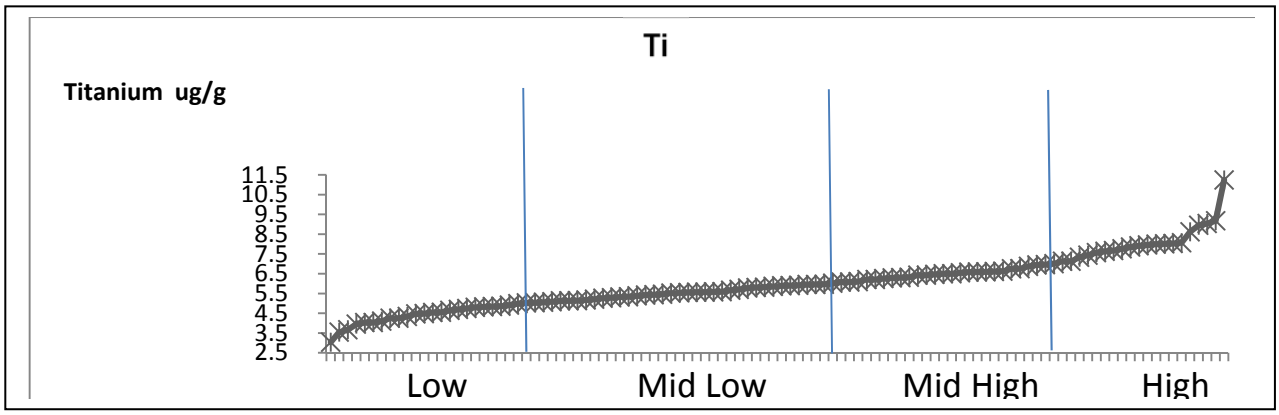

Figure 2. Distribution of fecal Ti concentration by treatment group (P<0.01) .

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FALL PASTURE QUALITY FOR COWS IN MID-PREGNANCY HAS MINIMAL EFFECTS ON OFFSPRING GROWTH

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ABSTRACT: The impact of pasture quality during the autumn grazing period on offspring of cows calving in late winter was studied over 4 years. Each year, between 26 and 50 cows, with an average calving date of February 12, were allocated to one of two treatments replicated in two pastures for the autumn grazing period. Cows grazed either pastures of seeded forage (26 ha each; average CP = 10.2% DM basis) from which a harvest of hay had been removed followed by flood irrigation in August or native rangeland pastures (71 or 90 ha; average CP = 6.7%). Cows grazed from about September 28 to November 19 at which time they were moved to drylots and fed a corn silage-based diet until calving. After calving, cows were fed hay and/or supplement until native rangeland forage was adequate to support production. Analysis of variance was conducted using a General Linear Models procedure to evaluate year, treatment and year by treatment interactions with age of dam and birthdate used as covariates and pasture within treatment as the experimental unit. Pre-weaning growth and 190-d adjusted weaning weights of calves did not differ due to type of pasture grazed in autumn. Pre- weaning ADG and 190-d adjusted weaning weight were 1.10 ± 0.01 kg/d and 245.8 ± 2.0 kg for heifers and 1.16 ± 0.01kg/d and 260.4 ± 2.1kg for steers. However, a tendency ($P < 0.08$) for a treatment by year interaction was observed for preweaning ADG for heifers. No differences in post-weaning ADG or weight at about one year of age were observed for cow grazing treatment for either heifers ($P = 0.55$, $P = 0.67$) or steers ($P = 0.27$, $P = 0.22$). Heifers and steers gained 0.59 ± 0.01 and 0.96 ± 0.01kg/d, respectively post-weaning. At 360 days of age, heifers weighed 342 ± 3.1 kg and steer weighed 416 ± 2.9 kg. Nutritional quality of pastures grazed by pregnant late winter calving beef cows for two months in autumn did not affect weights or growth of offspring to one year of age.

Key Words: beef cows, calf growth, fall grazing, forage quality

Introduction

Nutrient intake during gestation has been shown to affect growth and health of offspring (Funston et al., 2013), yet questions remain about the extent and timing of restrictions along with specific nutrients involved in these effects. In the Northern Great Plains, rangeland forage quality in autumn can be low as the majority of plant growth occurs before July 1 (Heitschmidt et al., 2004). In a previous study at this location (Short et al., 1996), cow BW and condition score changes during autumn varied greatly by year for cows grazing native rangelands. Cows lost weight and condition score in 1 of 3 years. Therefore, we hypothesized that in some years, higher quality forage, such as from seeded pasture, may be of benefit. The objectives of the current study were to determine if grazing pregnant cattle on seeded pastures during autumn would provide any long term benefit to a beef production system.

Materials and Methods

This study was conducted between 2005 and 2011 at the Fort Keogh Livestock and Range Research Laboratory (**LARRL**) near Miles City, MT (46°222 N 105°52 W). The natural rangeland vegetation is a grama-needlegrass-wheatgrass (*Bouteloua-Hesperostipa-Pascopyron*) mixed-grass dominant rangeland (Kuchler, 1964). Climate is continental and semi-arid with an average annual rainfall of 338 mm, 60% of which is received during the 150-d, mid-April to mid-September growing season.

All research protocols used in this study were approved by the USDA, ARS, Fort Keogh LARRL Institutional Animal Care and Use Committee. In each of 4 years (2005, 2007, 2009, 2010), between 26 and 50 cows, with an average calving date of February 11 ± 10 d, were allocated to 1 of 2 treatments replicated in 2 pastures for the autumn grazing period. Cows grazed either pastures of seeded forage (26 ha each; average CP = 10.2%) from which a harvest of hay had been removed followed by flood irrigation in August or native rangeland pastures (71 or 90 ha; average CP = 6.7%). Forages in the seeded pasture included grasses (smooth brome, Altai wildrye, Russian wildrye and western wheatgrass) and legumes (birdsfoot trefoil, red clover, and alfalfa).

Cows grazed from about September 28 to November 19 at which time they were moved to drylots and fed a corn silage-based diet until calving. After calving, cows were fed hay and/or supplement until native rangeland forage was adequate to support production.

Cows were weighed and a body condition score (BCS; 1= emaciated to 9 = obese; Whitman, 1975) assigned at the beginning and end of the grazing period. Average age at weaning across years was 187 ± 5 d and all calf weaning weights were adjusted to a 190-d basis. After weaning, heifers were placed in drylots and fed a diet appropriate for management goals. To account for any calf loss, a calculation was made of weight of weaned calves per pregnant cow included in the grazing study.

During the grazing period, diet samples were collected using either esophageal (2005, 2007) or ruminally (2009, 2010) cannulated mature beef cows. Extrusa samples were frozen, lyophilized and ground before analysis. Samples for CP determinations were placed in a roller grinder for 12 h (Mortenson, 2003). Nitrogen was determined by combustion (CE Elantech, Inc., Lakewood, NJ) and CP calculated as 6.25 x N, and values were expressed on a DM basis. In vitro digestibility was determined by the method of Tilley and Terry (1963; IVOMD) for 2005 and 2007. In 2009 and 2010 in vitro true digestibility (IVTDM) was determined using a Daisy II Incubator system (ANKOM Technology Corp, Fairport, NY). Samples were subjected to in vitro incubation in the presence of ruminal inoculum for 48 h at 39°C. For IVTMD, incubation bags containing samples were removed at 48 hr and rinsed until effluent was clear. Duplicate bags were placed into ANKOM220 Fiber Analyzer (ANKOM Technology Corp), and NDF disappearance was determined. Bags were dried at 60°C for 48 h to determine DM. In vitro true digestibility was calculated from the residue remaining after refluxing in NDF solution (Goering and Van Soest, 1970)

In 2005, standing crop was sampled on topographic sites by clipping within three 0.1 m^2 frames for each site to ground level in mid-October and mid-November. Representative site were upland, hillside and bottom for rangeland and upland for seeded pastures. Samples were dried, ground and analyzed for CP and IVOMD.

Analysis of variance was conducted using a General Linear Models (SAS 9.3, SAS Inst. Inc., Cary, NC) procedure to evaluate year, treatment and year by treatment interactions with cow age and calving date used as covariates and pasture within treatment as the experimental unit. Calf sex was used a covariate in all calf pre-weaning data analyses. Pearson correlation coefficients were determined among measures (SAS 9.3, SAS Inst. Inc., Cary, NC). Logistic regression was used to evaluate the number of days heifer progeny were retained in the herd.

Results and Discussion

Standing crop samples collected in 2005 showed total forage available to contain an average of 4.3 and 7.2% CP and 53.6 and 55.9% IVOMD in rangeland and seeded pastures, respectively. The low protein concentration of standing forage suggested that rangeland forage quality might not meet nutrient demands of pregnant beef cows.

Analysis of extrusa samples indicate that CP concentration of diets for cows grazing both pasture types met minimum protein requirements early in the grazing period (NRC, 1996). Therefore, evaluation of clipped forage samples alone was inadequate to determine that diet quality would meet nutrient requirements of a pregnant beef cows grazing rangeland.

Crude protein concentrations of extrusa from seeded pasture was greater ($P > 0.05$) than that from native rangeland except for November 2005/2007, but digestibility was not ($P < 0.10$; Table 1). It is possible that the lack of difference in digestibility is related to the addition of nitrogen in the in vitro system (Blümmel et al., 2004), however, cow weight and body condition changes were not affected ($P > 0.10$) by forage type (Table 2) and reflect the lack of difference in digestibility of extrusa samples between pasture types. Extrusa quality suggests that cows were able to select diets that met their nutrient needs in mid-gestation.

Birth weight, pre-weaning growth and 190-d adjusted weaning weights of calves did not differ ($P < 0.10$) due to type of pasture grazed by their dams in autumn (Table 3). Pre-weaning ADG and 190-d adjusted weaning weight was 1.10 ± 0.01 kg/d and 245.8 ± 2.0 kg for heifers and 1.16 ± 0.01kg/d and 260.4 ± 2.1 kg for steers. A tendency ($P = 0.08$) for a treatment by year interaction was observed for pre-weaning ADG for heifers.

Pre-weaning weight gain of calves was positively related ($r = 0.38$, $P < 0.001$) to weight gain of cows during the grazing period, but this was not affected by the grazing treatment, however, birth weight was not ($r = 0.02$, $P = 0.78$). Weight gain of cows during grazing was negatively correlated ($r = -0.36$, $P < 0.001$) to weight of cows at the beginning of the grazing period. This likely indicates that heavier cows had higher nutritional demands for maintenance and gained less during grazing. Subsequently, these cows may have had lower energy reserves for milk production and pre-weaning calf growth. Although the correlation between initial cow weight and pre-weaning calf gain was negative, it was not significant ($r = -0.11$, $P = 0.18$).

No differences in post-weaning ADG or weight at one year of age for heifer progeny were observed for the grazing treatments (Table 4; $P = 0.55$ and $P = 0.61$, respectively), but there was a tendency ($P = 0.08$) for a treatment by year interaction. Weaning weight of the first calf from heifer progeny tended to differ by year ($P = 0.06$) but not treatment ($P = 0.61$; Table 4). Days that heifers were retained in the herd (up to December 31, 2012) tended to be less ($P = 0.06$) for heifers from cows that grazed native rangeland in autumn (Table 4). This resulted in less ($P = 0.03$) weight of calf produced per year over the lifetime of the female progeny of cows grazing native rangeland compared to those of cows grazing seeded pasture.

Rolfe et al. (2011) reported decreased December and pre-breeding weight of heifer progeny born from cows grazing native rangeland in western Nebraska without protein supplement during winter compared to cows receiving protein supplement or those grazing corn residue without supplement throughout the last third of gestation. Cows grazing winter range without supplement lost body condition during the grazing period, whereas cows in the current study all gained body condition (Table 2). Although there was an effect of year on BCS change, these changes were always positive for both treatments. Additionally, cows in the Nebraska study remained on their grazing treatment until calving. Neither the reproductive performance of the heifer progeny nor the performance of the steer progeny were affected by winter grazing treatment of the cow in the Nebraska study.

Several studies reported differences in progeny growth resulting from maternal diet, however, these studies kept cows on dietary treatment until calving. Other studies have

reported that realiminentation of nutrient-restricted animals before calving has resulted in placental compensation such that treatment differences in placental function were not observed near term (Zhu, et al., 2006, Camacho et al., 2011). Therefore, changing the diet from pasture to a corn silage based diet during the last 1 to 2 months before calving may have resulted in some compensation, if needed.

Implications

In conclusion, cows on pastures of differing quality selected diets that allowed them to stay in positive nutrient balance throughout the grazing period. Therefore, nutritional quality of pastures grazed by pregnant late winter calving beef cows for 2 months in autumn did not affect weights or growth of offspring to 1 year of age. However, it appears that access to higher quality forages during this period maybe beneficial for retention of the female offspring kept for replacements.

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Table1. Crude protein and digestibility of extrusa[1] samples collected by mature beef cow grazing seeded pasture or native rangeland in the autumn.

Item	Month	Native Rangeland	Seeded Pasture
CP, %			
2005/2007	October	6.8±0.6[a]	11.6±0.6[b]
	November	5.6±0.5	7.9±0.5
2009/2010	October	8.7±0.2[a]	14.1±0.2[b]
	November	5.6±0.2 [a]	7.8±0.2 [b]
IVOMD[3]			
2005/2007	October	66.5±1.7	72.3±1.7
	November	68.1±1.5	66.8±1.5
IVTD[4]			
2009/2010	October	77.2±3.2	79.5±3.2
	November	70.5±3.5	71.7±3.5

[1] Extrusa was collected by esphogeally cannulated mature cows in 2005 and 2007 and by ruminally cannulated mature cows in 2009 and 2010.
[ab] Means with different superscripts between treatments differ, P < 0.05
[3]In vitro organic matter digestibility (Tilley and Terry, 1963)
[4]In vitro true digestibility (Goering and VanSoest, 1970)

Table 2. Weight and body condition score of beef cows grazing seeded pasture or native rangeland during the autumn.

Item	Pasture Type		*P*-value		
	Native Rangeland	Seeded Pasture	Treatment	Year	Treatment by year
Weight change, kg	60.8±4.5	64.9±4.5	0.576	0.040	0.645
BCS Change	0.33±0.06	0.31±0.06	0.841	0.425	0.668

Table 3. Weight and gain of steers and heifers born from cows grazing either seeded pasture or native rangeland for 2 months during mid-gestation.

Item	Pasture Type		*P*-value		
	Native rangeland	Seeded Pasture	Treatment	Year	Treatment by year
Birth weight, kg	38.0± 0.3	38.0±0.3	0.984	0.073	0.285
Adjusted 70-d weight, kg	110.8±1.8	108.7±1.7	0.486	0.059	0.457
Adjusted 190-d weight, kg	251.3±2.1	250.8±2.1	0.884	0.066	0.848
Preweaning ADG, kg/d	1.12±0.007	1.12±0.007	0.953	0.033	0.631
Kg calf weaned per pregnant cow[1]	228.6±5.1	242.2±5.1	0.196	0.157	0.752

[1]Unadjusted for calf sex

Table 4. Postweaning performance of heifers born from cows grazing either seeded pasture or native rangeland for 2 months during gestation

Item	Pasture Type		*P*-value		
	Native rangeland	Seeded Pasture	Treatment	Year	Treatment by year
ADG, weaning to 170 d post-weaning, kg/d	0.59 ± 0.004	0.59 ± 0.003	0.550	0.007	0.078
BW at 1 yr of age, kg	344.3±4.0	347.4±3.4	0.614	0.063	0.512
Weaning weight of first calf, kg[1]	196.5 ± 5.3	200.9 ± 5.1	0.609	0.063	0.544
Days of retention in the herd[2]	1074 ± 213.1	1480 ± 214.6	0.059[3]	0. 167[3]	
Total kg weaned calf per cow per yr[1]	107.6 ± 3.6	137.4 ± 3.8	0.027	0.280	0.170

[1] Data from progeny of cows grazing in treatment years 2005, 2007, and 2009 only
[2] Data to December 31, 2012
[3]Probability for chi-square

EXTENSION

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BEEFSD: AN INTEGRATED AND INTENSIVE EXTENSION CURRICULUM FOR BEGINNING BEEF CATTLE PRODUCERS IN SOUTH DAKOTA

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ABSTRACT: The average age of beef producers in the US is 58 and increasing. A need exists to increase the opportunity and success for the next generation of beef cattle producers. Extension at SDSU and South Dakota Farm Bureau Federation partnered to address this by providing a 3-year educational program that assisted beginning beef cow-calf ranchers in South Dakota to become economically, ecologically, and socially sustainable producers. The goal was to present a curriculum that contributed to future agricultural production, land stewardship, and rural community viability. The learning objectives were to provide: (1) evaluation of alternative production systems, (2) an integrated understanding of the entire US beef cattle industry, and (3) development of individual cattle enterprise plans. The target audience was a group of 30 beginning beef cattle enterprises represented by 43 individuals (several couples and a pair of brothers participated). The curriculum was comprised of six major kinds of activities: (1) instructional workshops, (2) case studies of established successful producers using a variety of production systems and management practices, (3) evaluation of post-weaning performance of participants' calves, (4) mentoring from established beef ranchers and other industry professionals, (5) web-based interaction, and (6) travel study trips to learn about other segments of the beef cattle industry. Outcomes of the program were evaluated using surveys of the participants beginning at 12 months into the program and thereafter at 6-month intervals. Responses were primarily open-ended written answers, lending themselves to qualitative analysis. Responses indicated that meaningful outcomes have occurred. For example, when asked the impact of the program on their operation, one producer indicated: "I just had to tell you guys that our net worth has increased by $37,000. If we can continue to be diligent we can either keep 30+ bred heifers or operate on our own money by the end of the year." Other participants reported comparable impacts. In conclusion, the beefSD program has been successful at fostering positive outcomes for the beginning producer participants.

Keywords: Extension service, beef cattle, beginning ranchers, beginning producers

Introduction

The demographic of South Dakota producers, like other states, is changing. According to the 2007 USDA Census of Agriculture, the average age of beef producers in the US was 58, and there were nearly twice as many producers over 65 as under 45 years of age. Beginning producers will need to successfully replace these aging producers as they retire.

Our goal was to provide a curriculum to beginning ranchers that would equip them with the tools to make wise management decisions that would lead to economic, ecological, and sociological sustainability and in turn contribute to ongoing agricultural production, land stewardship, and rural community viability. Our objectives were to:

1. Provide knowledge, skills, and experience with production, business, financial, and marketing tools, including their use in the overall ranch system.
2. Provide case studies of alternative range-based beef cattle production systems so beginning ranchers understand potential opportunities and management requirements of alternative strategies.
3. Provide feedback to beginning ranchers with cow-calf herds about the post-weaning feeding and carcass performance of their calves, as well as calves from alternative production systems.
4. Provide beginning ranchers with mentors that were successful established ranchers and who may be older generation ranchers considering transfer of assets and management to beginning ranchers.
5. Provide opportunities to gain firsthand knowledge of production, business, financial, and marketing aspects of the other segments of the beef cattle industry, including the feedlot, packing, and retail sectors.

The primary purpose of this curriculum was to provide them with the knowledge of the entire beef industry and the skills to assess change factors that will influence their economic sustainability. Additionally, the development of networking between beginning ranchers and mentors might provide beginning ranchers with access to capital resources. To fulfill these goals, we conducted an intensive 3-year educational program to assist beginning ranchers in South Dakota.

Materials and Methods

The program was initiated in the fall of 2010 based upon a partnership of SDSU Extension and the South Dakota Farm Bureau Federation (SDFBF) to provide this educational program to 30 beginning beef cattle producers in the state of South Dakota. Because of the large size of the target area for potential participants and the desire to keep participant groups small enough to engender relationship-building and discussion, we divided participants into two core groups, one each located in the eastern and western parts of the state. This also reduced

time and travel demands for the participants. Most activities were conducted within each core group, but some activities, such as travel-study trips, were conducted with the groups combined. This was partly needed for cost control for travel, but was also used to provide opportunity for familiarity and networking across the small-group memberships.

Recruitment of participants

The primary audience that we recruited from were beginning ranchers (defined as those in agriculture production as a business for ten years or less) who were currently involved in a family operation, those who were starting out on their own, and newcomers who had a great desire to ranch, but did not have the family connection or current financial means to get started.

We conducted an application process to recruit and select beginning producers to participate in the program. We found these beginning rancher applicants by advertising the program through news media and by working with agriculture organizations and service industries including South Dakota Farm Bureau Federation, South Dakota Cattlemen's Association, South Dakota Beef Industry Council, South Dakota Stockgrowers Association, South Dakota Farmers Union, agricultural lenders, veterinarians, USDA agencies such as Farm Service Agency and Natural Resource Conservation Service, and other people that serviced agriculture producers. We also contacted our existing Extension clientele that were beginning ranchers. Potential applicants were directed to Extension offices and the SDFBF web site for more information and the application form for the program.

We also recruited eight established successful ranchers from western and central South Dakota to serve as case-study coaches (see below) and mentors. These established ranchers practiced sustainable management and were financially stable. We recruited these mentors from our existing Extension clientele to build on established good working relationships and used our knowledge of the quality of their management practices.

Activities

The curriculum employed six major educational components: 1) conventional classroom instruction, 2) local "case-study" ranch evaluations of alternative beef cattle production systems, 3) evaluation of post-weaning performance of participants' cattle, 4) personalized interaction with mentors and a management advisory team, 5) interactive "web-based" learning experiences, and 6) multi-day travel-study trips.

Instructional Workshops. Workshops consisted of two-day meetings that included two major components: classroom instruction and ranch case studies. The specific location of each workshop changed for each session so that it was near the case study ranch for that session. Classroom instruction covered livestock, natural resource, marketing, business, financial, legal, and risk management tools for beginning ranchers. The livestock production and natural resource management curriculum included nutrition, range management, genetics, reproduction, animal health, and other topics. Utilization of cattle and pasture record keeping to benchmark performance and analyze management needs and opportunities was included. To develop financial management skills, each participant prepared balance sheets, cash flows, and determined unit cost of production during the program. Marketing was a major focus and was integrated in each meeting. Participants received the knowledge and skills to complete and implement business and marketing plans by the end of the project with guidance from their management advisory team. An estate-planning component was included that included both the beginning ranchers and the older generations of their families. Workshops also included components on communication and human relations.

The second component of the instructional workshops was case studies of four production systems that were evaluated throughout the entire project, including: 1) retained ownership through backgrounding of calves, 2) retained ownership to slaughter, 3) seedstock production, and 4) grass-fed production. Eight established ranchers (four per core group, one for each production system) served as case-study coaches, hosted tours of their operations and lead discussions about ranch history, their management philosophy and style, and decision-making criteria. The selected case study coaches were active leaders within their communities as well as state and national organizations; which supported the importance of developing community and organization leadership by beginning ranchers. Beginning ranchers spent part of a day at each operation as part of four of the 2-day workshops. Beginning ranchers were provided with background information related to each production system during the classroom session before and after the tour.

Post-Weaning Calf Performance Evaluation. Most South Dakota beef producers market weaned calves as their end product through a local auction market with minimal knowledge of the remaining production stages necessary to produce meat products and no knowledge of how their calves perform post-weaning. It was important to this program that beginning ranchers come to understand the entire beef production system in order to enhance their opportunity to adapt to accelerating business change in agriculture. It was also important that they learn the value of knowing their cattle's post-weaning performance. Beginning ranchers were asked to place up to five animals each into a post-weaning performance evaluation program. Participants received feeding performance (ADG, etc.), economic data (cost of gain, net income, etc.), and carcass data (carcass weight, quality and yield grade, etc.) on their cattle. A trip was made to visit the feedlot to see the cattle near the midpoint of the feeding period. We anticipated that some participants would be looking to enter cattle production, but not yet own a cow herd. We bought 12 head of cattle for them to follow through the feeding and harvesting process. These cattle were different biological types than those owned by the participants so we could demonstrate how these differences influenced finishing and carcass performance.

Mentoring. Mentoring was a major key to beginning rancher success during this project. Beginning ranchers developed personal relationships with case-study coaches and mentors during workshops, by exchange of information

through web-based technology, and during travel-study trips. All case-study coaches expressed enthusiasm to serve as mentors and assist beginning ranchers.

Instructors assisted beginning ranchers to form and utilize management advisory teams. Each participant established an individualized team with membership chosen to meet their specific needs. Teams consisted of four or five agriculture professionals such as mentors, agriculture organization staff, agriculture lenders, feed suppliers, marketing agents, federal agency personnel, lawyers, estate planners, and/or veterinarians.

Beginning ranchers hosted at least two ranch visits by members of our project team. The first ranch visits focused on assisting ranchers in conducting an inventory of their resources and determining goals. The second ranch visit focused on options to improve profitability and formation of management advisory teams.

Web-Based Interaction. Between face-to-face workshops, participants were expected to interact using web-based networking activities designed to help them continue to learn. A private group on Facebook was used as an online platform for participants to maintain an ongoing conversation about beef production. This allowed an informal exchange of ideas among participants (beginning ranchers, project team members, and case-study coaches). Each of them was expected to be interactive in the social group. A web presence was hosted on the SDFBF web site that had both a public portion and a login site for beefSD members. The public portion was intended to provide educational materials for both beefSD participants and the general public. This included things such as educational YouTube videos and podcasts. The login portion provided information that might be personal, announcements of beefSD activities, polls of participants, etc. Information presented through the web increased as the project proceeded to reduce travel to face-to-face meetings.

Travel Studies. Three trips were conducted to expose participants to marketing tools and opportunities, as well as post-weaning segments of the beef cattle industry. The first trip exposed participants to the breadth of marketing opportunities, and included aspects of cattle and meat production. Stops were made at farmer-feeders en route to Chicago to understand the characteristics of cattle feeding in the upper Midwest. In Chicago, participants visited the Chicago Mercantile Exchange. They also visited local farmer markets and other novel local marketing outlets in Chicago to experience alternative product (grass-fed, natural, and organic) marketing. Thus, participants were exposed to local purchasing opportunities of large city consumers. This included visiting a Whole Food grocery store, a local wholesaler of Prime quality beef, local farmers' markets, and an upscale white tablecloth restaurant. In these markets, the beginning ranchers were required to interact with and interview customers to gain an appreciation for the philosophies and motivators of their food-purchasing decisions. They gained an appreciation for potential product differentiation to add value through alternative markets.

The travel study trip during the second year focused on post-weaning production including large commercial feedlots, a processing plant, and meeting with cattle industry representatives (i.e., Cattle Fax). This trip was south of South Dakota into Colorado, Kansas, and Nebraska. Additionally, while in Colorado, arrangements were made for Temple Grandin to demonstrate facilities and procedures to provide low-stress treatment of cattle during handling and harvesting processes. Travel continued to Denver where participants met at the Beef Industry Council Culinary Kitchen and Cattle Fax to learn about the services they provide for cattle producers. This trip included tours of three different commercial feedyards and a large packing plant in Kansas, an elite seedstock producer, and a replacement heifer development facility, emphasizing the size and scope of the beef cattle industry.

By the third year, we expected that all participants would have completed and initiated their individual production and management plans. They should have an understanding of their knowledge and skills, and should also have a sense of further information they wanted or gaps in their knowledge base. At this point, the participants determined the destination(s) of the third travel-study trip to meet their needs and goals. They chose Washington DC to determine how government policy interacts with beef cattle production and the National Cattlemen's Beef Association meeting at Tampa, Florida.

Evaluation Plan

Evaluation was a continuous process throughout the project. The project team engaged consulting services of an evaluation professional to assist with assessment of ongoing progress towards desired outcomes. We conducted three surveys of the participants. The first (in 2011) was to determine baseline knowledge levels and to learn areas (production, business, financial, marketing, etc.) wherein they were most interested in gaining knowledge, skills and abilities. The second and third surveys were conducted during 2012 (at 12- and 18-month points in the timeline of the project) to determine progress toward impacts and outcomes.

Outcomes were also assessed by one-on-one interviews (during ranch visits or through interaction on social media) with individual participants. Mileposts, such as the completion of operational inventory, assembly of a professional management advisory team, and completion of business plans, financial statements, and marketing plans were evaluated during interviews.

Results and Discussion

Recruitment

We received 58 complete applications. We had originally planned for 30 individuals, but immediately upon advertising the program, we received inquiries from many couples and siblings about whether they could apply together. We adjusted our plans to include 30 beginning enterprises with up to 2 participants from each. Ultimately, we filled our goal of 30 enterprises, which included 14 individuals, 15 couples, and a pair of brothers.

Activities

Instructional Workshops. Seven multi-day workshops were completed, including: 1) case study of

retained ownership to backgrounding, 2) case study of retained ownership to slaughter, 3) case study of grass-finished production, 4) case study of seedstock production, 5) SD Grazing School (A joint-sponsored 3-day workshop by SDSU Extension and the SD Grassland Coalition), 6) BEEF2020 (A carcass and meat evaluation course conducted annually at the SDSU Animal Science Meats Laboratory), and 7) a cow-calf management workshop.

Post-Weaning Calf Performance Evaluation. Participant calves were fed at Darnall Feedyard near Harrisburg, NE. All participants received feedlot performance and carcass data. Participants had the opportunity to participate in market risk protection through hedging. Participants were hosted at Darnall Feedyard for a mid-feeding period meeting wherein they saw their cattle and participated in a mini-symposium, and a final meeting wherein they received their final report.

Mentoring. Ranch visits were completed and management teams were formed.

Web-Based Interaction. The Facebook private group has been very active. The project team members expected they would need to "seed" ideas into the conversation to stimulate discussion, but the participants have engaged actively without our stimulation. The login portion of the web site hosted by SDFBF has worked very well for announcements, polls, and other programming interactions. However, our presence on the public side has been limited. We delivered a four-part webinar series on Strategic Planning during Year 2. While it served its purpose for us to convey the material, it was not very satisfying because the participants were reticent to be interactive in the web-based setting. They were much more comfortable and talkative when in face-to-face settings.

Travel Studies. As expected, all 3 trips were highly successful and very well received and attended by the participants. The goal of having participants become aware of all segments of the beef cattle industry and their interactions was achieved.

Evaluation

Feedback from the surveys has been very positive. Excerpted outcome quotes by participants on the most recent survey related to our 6 major activity categories include**:**

Instructional workshops: "Made me come to the realization that I'm raising BEEF not cattle. Should be a requirement to attend this if you are going to mate cattle. Retrieved all carcass data on our home raised steers. Now comes to the culling of dams and sires of those that don't make the cut to improving the herd. Had no idea that 10 days too early or too late of harvest date could affect carcass quality that dramatically."

Case studies: "We have learned that networking is crucial. Being able to connect with someone who has more experience in this industry, ask them questions, and learn from their experiences is invaluable. We have also found that there is no one-right-way to be a cattle producer. There are many ways to be profitable in this industry if you are willing to take a few risks and try something new."

Calf post-weaning performance: "I have a better understanding of the costs involved in retaining ownership at a feedlot. I plan to use this experience when deciding what to do with my calves this fall." Another stated "I took what I thought was the bottom end of my calves to Darnall's Feedlot to get a baseline and I was very surprised. We had not been breeding for any carcass traits and we graded choice on our cattle. This baseline gave me the incentive to AI our entire herd this spring to improve on our carcass grade."

Mentoring: "I find that decision making whether it be about grass management, marketing or genetic selection is easier because I have the tools to do so and mentors to contact for further consultation if needed."

Web based training: "My strategic plan is lacking because it was not a real priority to me nor could I see a major benefit. My easy plan was to deal with each situation on a case by case basis. However, now that we are in a major drought, I realize the need for such a strategic plan. I plan to work on a solid plan starting with the information I am currently gaining."

Travel study trips: 1st quote; "Being able to see the production line at the Tyson plant was phenomenal. Meeting Temple Grandin and hearing her explain her designs face-to-face and then seeing how her designs were being used in virtually every cattle handling situation that we saw thereafter was amazing. It was such a unique experience to be able to see nearly every level of the beef industry all in one trip. We watched cattle go from feedlots, to kill floors, to freezers, to a culinary kitchen, and then to our plates. The "aha" moment of this trip (and really the entire beefSD experience) is that the beef industry is so, so much bigger than the cows in our pastures." 2nd: "The Culinary Kitchen presentation was really interesting. I plan to visit with my butcher about the possibility of trying some new cuts on our own beef, or maybe offering some of them to our freezer beef custom orders. I also plan to give my customers more than just a box of beef, but also try to give them some information on preparation of the meat through the beefitswhatsfordinner.com website or the Cooking Beef With Confidence booklet."

Implications

This program has had a positive impact on the beef cattle enterprises and lives of the beginning beef cattle producers that participated in the program. Sustaining the program is very important and we have initiated the second class of beefSD.

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SAFETY OF A VACCINE FOR EPIZOOTIC BOVINE ABORTION

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ABSTRACT: A study was conducted with oversight from the United States Department of Agriculture (USDA) to evaluate safety of a candidate vaccine for epizootic bovine abortion (EBA; also known as foothill abortion) as one component necessary in licensing. A secondary objective was to evaluate field efficacy. A total of 102 unbred heifers housed at University of California's Sierra Foothill Research and Extension Center were randomly assigned to be inoculated with either placebo (saline; *n=51*) or EBA vaccine (*n=51*) 28 d prior to breeding. Injection sites were shaved to facilitate reaction observations. Heifers were observed daily for 21 d for changes in overall health following vaccination. The injection sites were visually examined and palpated by a trained technician at 2, 7, 14, 21, 23, 30, 56 and 85 days post-vaccination (DPV); reactions were measured, described, and photographed. No morbidity or mortality was recorded during the observation period. Injection site reactions were noted in a total of 19 vaccinated units. Reactions were limited to swelling <6 cm in size. The first reactions were noted at 21 DPV with most resolving by 56 DPV and none noted by 85 DPV. No reactions were noted in the control animals. Heifers were bred by artificial insemination (AI) followed by 90 d of natural service. Rectal palpation for pregnancy was conducted 173 d post-AI and there was no difference between treatments (90.2% conception rate for each group). Efficacy could not be evaluated as none of the heifers in this study aborted fetuses suffering from EBA. Data from this study suggest the vaccine is safe. While localized reactions were noted in 37% of the vaccinated animals, they were mild and would have gone unnoticed had the sites not been shaved and palpated. No apparent negative impact upon conception was observed. Additional field trials are in progress in eight commercial herds in California and Nevada with no adverse reactions being noted to date. This study represents just one of many trials that will ultimately be required to obtain USDA licensure for this experimental vaccine but preliminary results suggest the vaccine is safe for use.

Keywords: Epizootic bovine abortion, reproductive diseases

Introduction

The realization of healthy offspring from each pregnancy is the basis for the economic well-being of the beef industry. Epizootic bovine abortion (**EBA**), also known as foothill abortion, is an infectious fetal cattle disease associated with late-term abortions or premature births in California, Oregon, and Nevada. The tick vector, *Ornithodorus coriaceus,* is the only known biological vector of the infectious bacterium that causes EBA. Foothill abortion is considered the leading cause of economic loss due to term abortions in beef cattle in California. Losses for individual producers have not been well documented; early reports were as high as 60% in naïve cattle (Stortz et al., 1960). Verbal communication with producers suggests that losses can approach 90% when naïve pregnant cattle are introduced into tick habitat.

Producers are limited in their ability to manage EBA. Current methods of controlling external parasites are ineffective against *Ornithodorus coriaceus*. Some producers in EBA areas have shifted breeding seasons and changed pasture rotations in attempts to reduce exposure of naïve cattle during the window of susceptibility which is considered to be approximately 50-140 days gestation; an additional approach is to naturally expose sexually mature naïve heifers to tick habitat prior to breeding to develop a degree of immunity.

Foothill abortion was described in 1956 by Howarth et al. but is believed to be the same syndrome reported in 1923 (McKercher et al., 1963). The causative etiologies of EBA have been mistakenly thought to be viruses, *Chlamydia, Borrellia,* and a spirochete (reviewed by Stott et al., 2002). A novel deltaproteobacterium was identified as the etiologic agent in 2005 (King et al). Efforts to develop a vaccine were initially thwarted by the inability to propagate the bacteria using synthetic media or cell cultures. Development and testing of a live bacterial vaccine became feasible with the development of a mouse model (mice with severe combined immunodeficiency) for propagating the bacteria *in situ* (Blanchard et al., 2010).

The objectives of this study were to evaluate safety of the candidate live bacterial EBA vaccine as one of the requirements for licensing. A secondary objective was to evaluate field efficacy.

Materials and Methods

A total of 102 sexually mature but unbred predominantly Angus heifers were housed at University of California's Sierra Foothill Research and Extension

Center near Browns Valley. Animal treatments were randomized as follows: a random number (generated using Excel software) was assigned to each unit's ear tag number, sorted based on the assigned random number and units divided into 2 groups based on their random number order. Observers were blinded to both treatment and treatment group. Heifers were inoculated with either placebo (*n*=51) or EBA vaccine (*n*=51). The experimental vaccine (Epizootic Bovine Abortion Agent Vaccine, Live Culture, unlicensed, USDA-CVB Product Code #1544.00) contains virulent bacteria; the product does not contain an adjuvant

Injection sites were shaved using electric clippers (minimum of 40 cm^2) and swabbed with 70% ethanol-soaked gauze pads. Either 1 mL placebo, consisting of sterile phosphate buffered saline, or 1 mL of vaccine was administered subcutaneously, using the tenting method, with a sterile 3 ml disposable syringe and 20-guage by 2.54-cm needle. A new sterile syringe and needle was used on each animal. Heifers were observed daily for 21 d for changes in overall health following vaccination.

The injection sites were visually examined and physically palpated by trained technicians at 2, 7, 14, 21, 23, 30, 56 and 85 days post-vaccination (**DPV**). Adverse reaction evaluation was based upon definitions provided in USDA Veterinary Service Memorandum No. 800-204. All reactions were noted; those >1.5 cm were measured (length x width) and photographed with an identifying placard to document progression or regression of reaction.

Heifers were bred AI 28 DPV, followed by a 90 d period of natural service after a 40 d period of rest for a Fall-calving date. Rectal palpation and ultrasound for pregnancy determination was conducted 85 DPV with a second pregnancy determination by rectal palpation at 173d post-AI. Conception rates were determined using data from the second pregnancy examination.

For the first 132 DPV, heifers were kept on improved irrigated pasture with minimal exposure to potential vector habitat. Heifers were placed in native range pastures, where the tick vector had been previously identified, during the next 3 months, 133 DPV to 225 DPV. Animals were then removed from exposure areas and returned to improved irrigated pasture until they had calved.

After potentially being exposed to EBA vector, animals were observed daily from 226 DPV until parturition or abortion. Aborted fetuses were collected and evaluated for EBA using techniques described by BonDurant et al, 2006.

Results and Discussion

No morbidity or mortality was observed during the 21 d daily observation period in the control or EBA vaccinates.

Injection site reactions were noted in a total of 19 (37%) vaccinated units over the course of the study. The first reactions were noted at 21 DPV (n=2). Two days later reactions were noted in an additional 3 animals. Reactions were noted in 19 heifers within 30 DPV, with all but 3 resolving by 56 DPV. None were noted at 85 DPV. One animal had a reaction lasting from 21 DPV to 56 DPV, the longest observed. All five reactions observed 23 DPV were also visible 30 DPV.

Reactions were limited to swellings ranging in size from 1.5 x 1.5 cm to 5.5 x 3.5 cm. No abscesses, bleeding, ulcerations, granulomas or alopecia were reported and no indication the sites were painful. No reactions were noted in the control animals.

Injection site lesions or reactions may be caused by many factors: animal's sensitivity to vaccine, contamination, and vaccination-associated injury. Since there were no lesions in the control animals, the reactions are assumed to be due to the animal's sensitivity to the vaccine, not contamination or vaccination injury. No adjuvant was used in the EBA vaccine, thereby reducing the incidence and severity of immunologically-mediated lesions typically associated with oil-based adjuvants. Given the sharp increase in heifers with injection site reactions between 23 and 30 DPV, the authors hypothesize that the total number of vaccinated units with reactions would have been higher had observations been conducted on a bi-weekly or weekly basis after 30 DPV. The prolonged period of injection site reactions were first noted in this study; previous observation periods were limited to 21 days and no reactions had been noted. Studies are underway to better define the extent and duration of reaction in vaccinates. While these mild reactions do not appear to pose a health risk, they do suggest animals are responding to the bacterial challenge.

Rectal palpation to determine the overall conception rate in the herd was conducted 173 d post-AI and there was no difference between treatment groups (n=46 pregnant units / group). This is important to note since the vaccine is against a disease which induces abortions. The vaccine is designed for use in open naïve heifers and/or cows prior to breeding. The vaccine has the capacity to infect the fetus with subsequent development of EBA; therefore it should not be administered to pregnant animals. The lack of difference between control and EBA vaccinates suggests the vaccine and associated immune response is not detrimental to conception when administered 28 d prior to the breeding season.

The sample size is insufficient to address possible post-conception losses; data is being accumulated in multiple herds to evaluate this potential safety concern. The possibility that viable bacteria may remain in the cattle for up to 60 days (based on injection sites reactions) has prompted researchers to extend the period between vaccination and breeding to a minimum of 8 weeks in succeeding studies as an added precaution to ensure vaccine safety.

Efficacy could not be evaluated as none of the heifers in this study aborted fetuses suffering from EBA. The soft-bodied tick *Ornithodoros coriaceus*, which transmits the disease through its bite, requires ambient temperatures to be warm and dry in order to activate the tick's metabolism. The opportunity for infection (based on gestational age) was primarily during the cooler, wetter spring months when tick activity was not at its peak, thus reducing the chances of infection.

Additionally, the ticks are required to be hungry and wildlife activity could have been such that they were the main feeding source for ticks, thus reducing the need for feeding on cattle.

Impacts

Data from this study suggest the vaccine is safe. While localized reactions were noted in 37% of the vaccinated animals, they were mild and most would have gone unnoticed had the sites not been shaved and palpated. No apparent negative impact upon conception was observed. Additional field trials are in progress in eight herds in California and Nevada and have not yet identified any safety concerns as regards to adverse reactions, either to general health or in severe vaccine injection site reactions. This study represents just one of many trials that will ultimately be required to obtain USDA licensure for this experimental vaccine and to reduce the economic impact of EBA on beef producers in the western United States.

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EFFECTS OF EWE EFFICIENCY CLASSIFICATION AND METHOD OF DIET PROCESSING ON SUBSEQUENT FORAGE BASED GROWTH TEST

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ABSTRACT: Residual feed intake (RFI) can identify sheep that grow more efficiently during a grower test; however, to our knowledge it is not known if RFI classification predicts growth efficiency on forage based diets in subsequent feeding trials. Our objectives were to determine intake and growth rates of ewes divergently classified for high and low RFI fed either chopped or pelleted alfalfa hay (2 x 2 factorial). To establish efficiency classification, forty six ewe lambs had unlimited access to a pelleted grower diet for 63 d. Residual feed intake (RFI) values were calculated as the difference between actual and predicted DMI based on metabolic BW and ADG. Thereafter, half of the ewe lambs that had the most positive (**Inefficient**; n = 16) and most negative (**Efficient**; n = 16) RFI values were placed into one of two pens that had ad libitum access to either pelleted or chopped alfalfa hay. Ewe lamb BW, LM area (LMA), and 12th rib back fat (FD) were measured at the beginning and end of the 56 d feeding period. No interactions between RFI classification and method of hay processing were detected ($P \geq 0.17$) for all measurements. Pelleted hay increased ($P < 0.01$) ewe lamb BW, DMI, ADG, G:F, LMA, and FD compared to chopped hay. Inefficient ewes had greater DMI and RFI values ($P < 0.01$) than efficient ewes; however, no difference ($P \geq 0.46$) were detected between RFI groups for ADG, G:F, or LMA. Inefficient ewes tended ($P = 0.06$) to have greater final FD than efficient ewes. Within hay treatments, numerical differences for DMI and RFI values between efficiency groups were twice as large for ewes on the pelleted hay compared to chopped hay diet. This data suggests that ewe efficiency as determined by RFI is repeatable on subsequent forage based trial; however, difference in intake and efficiency are more apparent if forage is pelleted.

Key Words: residual feed intake, sheep, intake, growth

Introduction

Feed costs account for the largest input cost in livestock production systems and identification of animals that require less feed for normal production would clearly increase productivity. Residual feed intake (RFI) is the concept of developing an alternative feed efficiency measurement that is independent of growth traits and was first proposed by Koch et al. (1963). Residual feed intake is the difference between actual feed intake and predicted feed intake based upon maintenance of BW and ADG by linear regression. Numerous research efforts have shown that there is considerable individual animal variation in RFI in cattle (reviewed by Archer et al., 1999 and Herd et al., 2003) and sheep (Snowder and Van Vleck, 2003 and Cammack et al., 2005). However, most RFI testing has been conducted post-weaning on medium-to-high energy diets and has been related to potential feed savings in the feedlot (Snowder and Van Vleck, 2003). Research from our laboratory found no phenotypic correlation between RFI that was determined on a pelleted grower ration and RFI that was determined on a chopped hay diet at maintenance (Redden, 2011); however, we could not attribute the lack of RFI relationship to diet or rate of growth. Therefore, our objective was to determine ewe lamb intake and growth efficiency of ewe lambs classified into high and low RFI groups on pelleted and chopped alfalfa hay.

Materials and Methods

Forty six Targhee ewe lambs (BW = 42 ± 5 kg; 6 month-of-old) were selected randomly from the Red Bluff Research Ranch 2010 spring-born lamb crop. Use of animals was approved by Montana State University Animal Care and Use Committee.

In Trial 1, ewe lambs were transported from the Red Bluff Research Station to the BART Farm in Bozeman, MT. Ewes were housed together in one pen (10 X 10 m) with 4 GrowSafe pods (GrowSafe Systems Ltd., Airdrie, AB, Canada) and false bottoms were constructed to modify GrowSafe beef cattle stanchions and feed bunks for sheep. Ewes were given ad libitum access to a pelleted grower diet (Table 1) and water. Initially, all feed bars were removed so that multiple ewes could eat at the same time. After 1 wk, feed bars were placed in the feed trough so that only one animal could have access to the feed bunk at any given time. After a 2 wk acclimation period, individual feeding events and feed disappearance recordings were initiated. Feed samples were collected weekly and stored for later analysis. Ewe BWs were measured weekly with two consecutive day weights recorded at the start and end of the 56 day test. Longissimus muscle area (LMA) and 12th rib backfat depth (FD) were determined via ultrasound by an experienced technician at the beginning and end of the RFI Trial to estimate lean muscle and adipose deposition, respectively. Growth rates of individual ewes were modeled by linear regression of 7-d BW by using a PROC GLM procedures of SAS (SAS Inst. Inc.), and regression coefficients were used to compute ADG, initial and final BW, and metabolic BW (**MBW**; midtest $BW^{0.75}$) as described by Lancaster et al. (2009). Similarly, LMA and BF were modeled by linear regression to compute modeled LMA and BF deposition rates. These rates were used to

estimate the effects of lean and fat deposition on RFI. Expected feed intake was calculated for each ewe by regressing actual feed intake against MBW and ADG during the trial (Koch et al., 1963). Residual feed intake was calculated by subtracting actual intake from expected intake. In the model, MBW and ADG accounted for most of the variation in DMI ($P < 0.0001$) and the R-Square of the model was 0.84. Ewe LMA and FD change did not significantly account for variation in the model ($P = 0.72$ and 0.24, respectively), nor did they improve the R-Square (0.85) and were subsequently omitted from the model. In contrast, Knott et al. (2008) reported that ultrasound estimates of lean and fat should be included in the RFI determining model because it slightly improved the R-square of the model. However, authors noted that both measurements were not significant in the model and in most cases it would be removed. After the conclusion of Trial 1, ewes were removed from the GrowSafe testing facility and returned to the Red Bluff Research Station.

In Trial 2, thirty two ewe lambs that had the largest positive and negative RFI values were assigned to high and low RFI groups, respectively. In March 2011, the high and low RFI ewes were returned to the GrowSafe testing facility. Half of the ewes from each RFI group were placed in one of two pens that contained two GrowSafe feed bunks with ad libitum access to alfalfa hay (Table 1). One load of alfalfa hay was either chopped or pelleted and provided to one of two pens exclusively. Feed intake measurements were collected similar to Trial 1. Ewe BW were measured weekly with two consecutive day weights recorded at the start and end of the 56 day experimental period. Growth rates of individual ewes were modeled by linear regression of 7-d BW as described previously. Ultrasound LMA and FD were determined at the beginning and end of the limit-fed experiment to estimate lean muscle and adipose deposition, respectively.

Results and Discussion

We sorted the ewes in Trial 1 into efficient and inefficient groups based on RFI values. Data from Trial 1 are reported for the 32 ewe lambs that were used in Trial 2 (Table 2). By design, low RFI ewes required 0.21 kg less of feed than high RFI ewes to achieve a similar rate of gain and maintain a similar body weight during the RFI testing period. During this period, ewes in the low-RFI group consumed 9% less feed ($P < 0.01$) than ewes in the high-RFI group, while ewe BW and ADG were similar ($P > 0.75$). There were no detectable differences ($P > 0.19$) among RFI groups for initial and final LMA or FD during the RFI testing period (Table 2). Ewe lamb BW differences were not expected because the RFI model accounts for difference in ADG and MBW, similar to previous work from our laboratory (Redden et al., 2011). In contrast, lean and fat deposition has accounted for some variation in RFI models for beef cattle (Herd and Arthur, 2009) and sheep (Knott et al., 2008); however, authors comment that variation in body composition was small relative to other factors that predicted intake.

For commercial application of this technology, it is recommended that animals are grouped into high, medium, and low categories for selection purposes. Sheep producers may use this technology by selection of replacement breeding animals from the highly efficient category or selection against highly inefficient animals. The following data set provides information on how animals from each category will perform in free choice feed setting with pelleted or chopped forage.

In Trial 2, no interactions between RFI classification and method of hay processing were detected ($P \geq 0.17$) for all measurements (Table 2).

Ewe lamb intake was 54% greater ($P < 0.01$) in ewes fed the pelleted hay compared to the chopped hay (Table 2). Similarly, Heaney et al. (1968) reported that pelleting increased digestible energy intake by 9, 36, and 114% for early, middle, and late matured alfalfa hay. Moreover, Heaney et al. (1968) reported that pelleting alfalfa hay only had a slight reduction in dry matter digestibility (2, 9, and 0% for early, medium, and late matured hay, respectively). Therefore, it came as no surprise that ADG, G:F, LMA, and FD were greater ($P < 0.01$) at the end of the trial from ewes fed the pelleted vs chopped alfalfa hay.

In the pelleted vs chopped hay trial, no differences ($P \geq 0.46$) were detected for initial BW, final BW, ADG, or G:F between RFI groups (Table 2). This is in agreement with Redden et al. (2011), who found no relationship between growth and feed efficiency traits determined as ewe-lambs fed a pelleted grower diet and as yearlings fed a chopped hay diet. In contrast to the aforementioned study, high RFI ewes (inefficient) had 24% greater feed intake ($P < 0.01$) than low RFI ewes (efficient). Within hay treatment groups, high RFI ewes had 28 % greater ($P = 0.02$) DMI than low RFI ewes on the pelleted diet; whereas, high RFI ewes tended ($P = 0.08$) to have 20 % greater DMI than low RFI ewes on the chopped hay diet. Similarly, high RFI ewes had 0.76 and 0.31 kg/d greater ($P = 0.02$) RFI values on Trial 2 than low RFI ewes on the pelleted and chopped hay diets, respectively.

No differences ($P \geq 0.23$) were detected for initial LMA and FD or final LMA between RFI groups. However, final FD tended to be greater ($P = 0.06$) for high than low RFI ewes. Similarly, Hafla et al. (2012) reported that low RFI bulls had 10% less BF than high RFI bulls. We attribute this difference to higher rates of adipose deposition as a result of higher DMI intake not represented by ADG. We feel that this difference in fat deposition was not seen during Trial 1 because the ewes were younger in a more lean growth phase. Selection for animals that deposit more or less adipose tissue during later stages of development could have positive and negative effects on market lambs and breeding sheep. One particular area of interest is the link between RFI and reproduction. In beef cattle, high RFI animals have been shown to have higher twinning rates and a shorter calving season (Basarab et al., 2007). Similarly, Halfa et al. (2012) reported that percent normal sperm was weakly correlated with RFI. Additionally, quality and yield grades of lambs are highly dependent on fat deposition. However, to our knowledge, no data exists that describes the effects of RFI selection on market lamb performance. In beef cattle, slight negative attributes have been reported from low vs high RFI steers (Baker et al., 2006), even though, ultrasound LM area and back fat thickness were

included in the model. This data warrants further research into the effects of age of lamb and diet used to collect RFI values and how this impacts posttest performance.

Conclusions

Selection of high and low residual feed intake values determined in 6 mo-old ewe lambs fed a pelleted grower ration identified animals that were ~10% more efficient during the test period. In a subsequent test on pelleted or chopped alfalfa hay, efficient ewes as determined on the previous trial had 24% lower intakes and similar gains to inefficient ewes. Intake differences were more apparent in ewe fed the pelleted diet. Inefficient ewes tended to deposit more fat than efficient ewes. Residual feed intake could be an important tool to reduce feed costs for sheep production; however, impacts of selection for efficiency traits on breeding sheep and subsequent market lamb performance should be established prior to commercial application of this technology.

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Table 1. Ingredient composition and chemical composition of feedstuffs.

	Diet or feedstuff[a]		
	Grower Pellet	Chopped Alfalfa	Pelleted Alfalfa
Ingredient, % as fed basis			
Alfalfa sun-cured	43.60	100	100
Corn	23.93		
Wheat middlings	15.00		
Soybean hulls	10.00		
Molasses cane	4.00		
Calcium carbonate	2.02		
Ammonium chloride	0.60		
Pelletizing agent	0.50		
Premixes[b]	0.35		
Chemical Composition			
DM, % (as fed)	88.5	91.1	86.8
CP, % of DM	15.8	15.5	15.8
TDN, % of DM	75.0	57.2	62.4

[a] Ewe lambs were allowed ad libitum access to the pellet diets.
[b] Contained 2,000 mg/kg Mo; Contained 44,000 IU/kg vitamin E; Contained 9 % Fe, 10 % Zn, 6% Mn, 3.4% I, and 0.03% Co; Contained 0.02 Se; 23700 IU/mg vitamin A and 2250 IU/mg vitamin D.

Table 2. Effects of RFI classification and method of forage processing on ewe lamb intake and growth performance

	Chopped Alfalfa		**Pelleted Alfalfa**			***P*-value**		
Item[1]	**Efficient**	**Inefficient**	**Efficient**	**Inefficient**	**SE**	**Hay**	**RFI**	**Hay*RFI**
No. of ewes	8	8	8	8				
Trial 1[2]								
Initial BW, kg	42.6	41.4	42.2	45.0	4.2	0.40	0.75	0.38
Final BW, kg	61.3	61.7	61.6	60.7	2.3	0.72	0.82	0.58
ADG, g	293.3	299.4	296.8	282.6	36.2	0.72	0.82	0.58
DMI, kg/d	2.21[a]	2.44[b]	2.22[a]	2.39[b]	0.13	0.75	<0.01	0.64
RFI, kg	-0.10[a]	0.11[b]	-0.10[a]	0.11[b]	0.04	0.97	<0.01	0.97
Initial LMA, cm^2	11.4	11.4	11.8	12.6	0.88	0.09	0.42	0.36
Final LMA, cm^2	17.9	17.4	17.4	16.9	1.12	0.34	0.41	0.88
Initial BF, cm	0.21	0.25	0.24	0.24	0.03	0.50	0.24	0.10
Final BF, cm	0.65	0.65	0.62	0.62	0.07	0.37	0.91	0.94
Trial 2[3]								
Initial BW	53.4	53.1	58.6	60.5	4.27	<0.01	0.72	0.63
Final BW	60.4[a]	61.4[a]	73.2[b]	73.2[b]	2.53	<0.01	0.68	0.73
ADG, g/d	71.2[a]	88.6[a]	299.8[b]	301.2[b]	45.1	<0.01	0.68	0.73
DMI, kg/d	2.00[a]	2.40[ab]	3.01[b]	3.85[c]	0.38	<0.01	<0.01	0.26
RFI, kg/d	-0.15[ab]	0.15[bc]	-0.38[a]	0.38[c]	0.31	1.00	<0.01	0.15
G:F, g:kg	26.1[a]	32.3[a]	99.1[b]	79.2[b]	18.3	<0.01	0.46	0.17
Initial LM area, cm^2	17.5	17.5	17.6	18.1	1.03	0.56	0.61	0.71
Final LM area, cm^2	17.1[a]	17.6[a]	20.5[b]	20.4[b]	1.2	<0.01	0.77	0.63
Initial BF, cm	0.48	0.51	0.40	0.45	0.06	0.06	0.23	0.77
Final BF, cm	0.42[a]	0.52[a]	0.72[b]	0.74[b]	0.06	<0.01	0.06	0.18

[abc]Means with different superscripts differ ($P < 0.05$)
[1]RFI = residual feed intake; BW = body weight; ADG = average daily gain; DMI = dry matter intake; G:F = gain:feed; LMA = longissimus muscle area; BF = back fat depth
[2]Trial 1 was conducted on a pelleted grower ration for 56 days and individual intakes were collected.
[3]Trial 2 was conducted on either pelleted or chopped alfalfa hay for 56 days and individual intakes were collected. Initial BW was added as a covariate for all variables to account for differences association with initial BW differences.

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SUPPLYING PECTIN TO CATTLE: A POSSIBLE WAY TO REDUCE CHOLESTEROL IN MEAT

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ABSTRACT: Probably, the main disadvantage of red meat consumption is its cholesterol content. Health authorities recommend reducing the intake of saturated fats and cholesterol. Previous studies in different animal species, including humans, have shown that cholesterol concentration in blood decreases if pectin is included in the diet. However, no studies have been reported on pectin supplementation to cattle and its possible effect in reducing blood cholesterol, an indicator of cholesterol reduction in striated muscles. The aim of this work was to explore the possible reduction of cholesterol and total fat absorption in meat animals when pectin is directly supplied to the duodenum. Steers were treated with pectin at three levels through a duodenal cannula.

Samples were taken daily during 5 days to analyze cholesterol in blood and total fat in feces. Blood cholesterol significantly decreased (P<0.0001) 15.6, 18 and 27% for increasing pectin concentration (0.004, 0.02, 0.01%, respectively), whereas excreted total fat significantly increased (P>0.0001) 10.5, 25.3, 19.9, 22.6 **%** at 0, 0.004, 0.02 and 0.01% pectin supply, respectively. It was concluded that pectin supplementation decreased blood cholesterol and fat absorption in feces. These results were an indication of reduced cholesterol deposition the striated muscle. Therefore, cholesterol-reduced red meat can be produced by supplying pectin to cattle in a protected form, such as encapsulated, allowing this carbohydrate to reach the duodenum where it is absorbed.

Introduction

Due to the high incidence of cardiovascular cases in Mexico, lipid content in foods is a major concern for health authorities and consumers' organizations. A number of studies have been focused on reducing cholesterol level and decreasing fat absorption in bovines, in order to produce more healthy meat. Commercial pectin supply have been a subject experiments in several animal species, nut not in bovines; it is assumed that the mechanism for cholesterol reduction by pectin is due to the presence of methoxy radicals, producing a viscous gel (Ershoff, 1962). Other authors report that viscosity increase in the small intestine reduces hypocholesterolemia, action due to pectin binding bile salts, allowing cholesterol expulsion in feces (Kay, 1977). This mechanism also reduces the risk of the onset of several types of cardiovascular diseases (Jackson, 2007). Lin (1957) found that pectin supply to rats increased fecal excretion of lipids, cholesterol and bile salts. The objective of this work was to study the effect of pectin supplementation in bovine diets on cholesterol concentration in blood and in total fat excretion in feces.

Methodology

Four Holstein steers, 462.5 kg average weight, with rumen and duodenal cannulas, were allocated on 2.42 x 3.74 m individual pens al Animal Nutrition Laboratory, Colegio de Postgraduados, Montecillo, Mexico. They were fed twice a day (7 and 19 hours) with alfalfa hay (basal diet), 9.6 kg DM approximately total daily consumption; and 0.4% chromium oxide as digestibility marker. The pectin daily consumption (38.41 g/day) was divided into two doses and supplied immediately after each feeding time thorough the rumen cannula.

The experiment was carried out thorough four treatments: 1. basal diet + chromium oxide; 2. basal diet + chromium

oxide + pectin low dose (0.004%); 3. basal diet + chromium oxide + pectin medium dose (0.01%); 4. basal diet + chromium oxide + pectin high dose (0.02%). Pectin percentages were based on the animal's live weight. Treatments were applied for four periods of 10 days adaptation and 5 days sampling; 400 to 500 mL rumen liquid was sampled from each steer. Fecal and duodenal sampling was carried out 1.5 h postprandial for 5 days. Blood samples were taken at before starting the experiment and at the last day by puncture at the vein coccigea, using Vacutainer needles and tubes containing EDTA.

Samples were taken to the laboratory in an icebox at 4ºC, and centrifuged at 3000 rpm for 4 min. The supernatant plasma and serum were checked for cleanliness and absence of hemolytic residues. The plasma was then stored at -20^{o} C until analysis. Fat extraction was carried out using Goldfish equipment (Labconco, New York) according to the AOAC method (1990). Cholesterol analysis was carried out in a HPLC Varian equipment (Australia) fitted with a Varian Pro Star pump and Galaxy software; a reverse phase C18 Symmetry column was used (15 cm length, 3.9 mm internal diameter, 5 μm particle size) under isocratic conditions. The experimental design was a Latin Square 4 x 4 (steers x treatments: control 0.004, 0.01, 0.02% pectin).

Results and Discussion

Non-significant differences were observed in nutrient digestibility and absorption from intake to feces (total digestibility) with respect to dry matter, organic matter, protein and NDF; however total fat digestibility decreased ($P<0.05$) with pectin infusion, even at the lowest dose (0.004%), as a possible result of higher pectin percentage covers most feed chime and prevents the action of gastric, bile and pancreatic juices, facilitating the absorption process. Pectin can also momentarily adhere to the vellocidades epithelium, preventing fat absorption.

Cholesterol concentration decreased as pectin in the treatment increased ($P<0.0001$, $r=-0.92$); the mechanism of pectin promoting hypocholesteronemia is not clarified, although it is suggested that pectin blocks cholesterol absorption or diminishes the enterohepatic circulation; in both cases, it interferes with the absorption of bile salts. These probable mechanisms have been studied in rat liver and plasma (Leveille and Sauberlich, 1988), in chickens (Erdman et al., 1986) and in humans (Jenkins et al., 1975; Rock and Swendseid, 1992). The results obtained in rat show that low pectin levels in plasma and liver are due to the inhibition of bile salt absorption and these, at the same time, reduce cholesterol absorption (Rock and Swendseid, 1992).

Conclusion

To the best of our knowledge, no results have been reported in bovines on the effect of pectin infusion on blood cholesterol and total fat excretion in feces. The present study showed that, in cannulated animals, when pectin in supplied through the duodenum, it interacts with fats and decreased cholesterol, probably due to a physical trapping in the high methoxyl pectin gel. However, it is possible that hydrophobic interactions also occur between pectin and lipids. Trapping and interactions prevents cholesterol absorption in the intestine and, as a consequence, accumulation in the animal's organism, producing a better healthier meat. Future studies will include the use of encapsulated pectin, avoiding its degradation before reaching the duodenum.

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THE ALVEOLAR-ARTERIAL OXYGEN PRESSURE GRADIENT OF PRE-WEANED BEEF CALVES IS NEGATIVELY ASSOCIATED WITH THE CROSS-SECTIONAL AREA OF THE LONGISSIMUS DORSI MUSCLE

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ABSTRACT: An alveolar-arterial oxygen (A-a O_2) pressure gradient is a measure of O_2 diffusion efficacy from lung alveoli into pulmonary blood. An A-a O_2 pressure gradient > 10 mmHg is an indicator of poor O_2 transfer. Gradients can be calculated from arterial blood-gas tensions. Variation in A-a O_2 gradients among calves may be explained by diffusion impairment, right-to-left vascular shunt or more commonly, ventilation-perfusion mismatching. Pneumonia has been associated with long-term detrimental effects on productivity, such as growth rate. This may be because cattle have very little pulmonary reserve. Therefore, lesions that reduce the gaseous exchange surface area or impede O_2 diffusion across the blood-gas boundary are likely to increase the A-a O_2 gradient. Skeletal muscle growth has a high O_2 requirement. *Longissimus dorsi* cross-sectional area is an indicator of total body muscling. We hypothesized that the A-a O_2 gradient is negatively associated with *l. dorsi* area. Beef calves, 102 to 280 days old, were randomly selected from two herds at 1,466 m (Herd A, n = 30) and 2,008 m (Herd B, n = 30) above sea level. Blood obtained from the coccygeal artery was analyzed using a handheld blood-gas analyzer. Height was measured from the ground to the iliac crest. *L. dorsi* area was obtained ultrasonically between thoracic vertebrae 12 and 13. Calves in herd A were sampled twice, 118 days apart. Complete sets of observations were analyzed using generalized estimating equations (n = 81). A-a O_2 gradients ranged from 2.3 to 43.6 mmHg with a mean of 23.0 ± 0.9 mmHg. Calf age was not associated with *l. dorsi* area when controlling for height ($P = 0.40$) and was eliminated from the model. *L. dorsi* area was negatively associated with A-a O_2 gradient ($P = 0.001$) when controlling for ranch ($P = 0.74$), sex ($P = 0.07$) and hip height ($P < 0.001$). An increase in the A-a O_2 gradient by 1 standard deviation (8.3 mmHg) was associated with a decrease in *l. dorsi* area of 1.3 ± 0.5 cm^2. It can be concluded that, in the calves sampled, inefficacious alveolar-arterial O_2 diffusion was associated with reduced *l. dorsi* muscle growth. This highlights the importance of minimizing the presence of pulmonary pathology in order to achieve optimal growth performance.

Key words: beef calves, muscle growth, oxygen

INTRODUCTION

Bovine respiratory disease (**BRD**) is a leading cause of morbidity and mortality (Loneragan et al., 2001). BRD has long-term detrimental effects on productivity. The occurrence of BRD in dairy calves less than 3 months old significantly reduces weight gain and first lactation milk production (van der Fels-Klerx et al., 2002). The presence of pulmonary lesions in feedlot steers has been associated with significantly reduced weight gain (Wittum et al., 1996). Cattle have limited pulmonary reserve due to their small total alveolar surface area available for gaseous exchange relative to O_2 demand (Veit and Farrell, 1978). The accumulation of pulmonary lesions may reduce the already limited gaseous exchange surface area. This may increase ventilation-perfusion mismatching or cause other pulmonary pathophysiological abnormalities that reduce the efficacy of O_2 transfer from the alveoli into the pulmonary circulation. The efficacy of O_2 transfer can be estimated from the alveolar-arterial O_2 (**A-a O_2**) pressure gradient. Efficacy of O_2 transfer is inversely related to the A-a O_2 gradient. Skeletal muscle is a major determinant of basal O_2 requirements (Zurlo et al., 1990). Therefore, efficacy of O_2 uptake may be a limiting factor for skeletal muscle growth. Live animal total muscling can be predicted ultrasonographically from the cross-sectional area of the *longissimus dorsi* muscle (Herve and Campbell, 1971). We hypothesized that the A-a O_2 gradient of pre-weaned beef calves is negatively associated with the cross-sectional area of the *longissimus dorsi* muscle.

MATERIALS AND METHODS

The Colorado State University Animal Care and Use Committee approved of the animal handling and sampling procedures prior to sample collection.

Study site

Calves from 2 herds in northern Colorado were randomly selected for the study. The same cohort of Black Aberdeen Angus calves from herd A was sampled on 2 occasions. Repeated measures were accounted for in the statistical analysis. Calves from herd B were born to red

Aberdeen Angus and Hereford composite cows bred to red Aberdeen Angus bulls. Due to herd management practices calves in herd B were tested only once (Table 1). All sampled calves were apparently healthy at the time of sampling and had not been previously identified as sick nor treated for respiratory disease.

Table 1. Herd, altitude, date of sampling, number of calves sampled and age

Herd	Altitude, m	Date	n[1]	Mean age ± SD, days
A	1,466	07/01/2011	30	103.5 ±16.0
		10/27/2011	28	249.7 ± 15.9
B	2,008	09/27/2011	30	126.1 ± 13.0

[1] number of calves sampled

The dams of calves studied were given a pre-breeding and pre-calving vaccination offering protection against *Bovine herpesvirus 1* (infectious bovine rhinotracheitis [IBR]), BVDV, *Bovine respiratory syncytial virus* (BRSV), and *Bovine parainfluenza virus 3* (BPIV-3). Calves were vaccinated against the same respiratory pathogens at 4-8 weeks of age and 2-4 weeks prior to weaning. Herd A used a modified-live vaccine and herd B used a killed vaccine on both cows and calves. Calves on both ranches were administered a killed vaccine at 4-8 weeks of age offering protection against: *Cl. chauvoei, Cl. septicum, Cl. novyi, Cl. sordellii* and *Cl. perfringens* Type C and D. Vaccines were given according to the manufacturer's instructions. Herd A takes ear notch samples routinely from all calves kept as replacement heifers for *Bovine viral diarrhea virus* (BVDV) enzyme-linked immunosorbent assay testing. Herd B tests suspect animals based on the advice of a veterinarian. No calves persistently infected with BVDV have been detected in either herd to date. Communal grazing does not occur. Salt licks are provided year-round. Growth promotants were not used. Calves were born with minimal assistance.

Alveolar-arterial oxygen pressure gradient

The A-a O_2 pressure gradient is an indicator of the efficacy of O_2 transfer from the alveoli to the pulmonary blood. An A-a O_2 pressure gradient > 10 mmHg is an indicator of poor O_2 transfer due to ventilation-perfusion mismatching, diffusion impairment or right-to-left vascular shunt (Lekeux, 1993). The A-a O_2 pressure gradient is calculated using the following formulae:

$$\text{A-a } O_2 \text{ pressure gradient} = P_AO_2 - PaO_2$$

$$P_AO_2 = FiO_2(BP - pH_2O) - (PaCO_2/R)$$

where P_AO_2 = alveolar O_2 tension (mmHg); PaO_2 = arterial O_2 tension (mmHg); $PaCO_2$ = arterial CO_2 tension (mmHg); R = respiratory exchange ratio (0.9); FiO_2 = fraction of inspired O_2 (0.21); BP = barometric pressure (mmHg); and pH_2O= water vapour pressure at body temperature (52.4 mmHg at 39°C).

A study of 7 cows at least 2 years old reported a mean respiratory exchange ratio of 0.91 (± 0.05) (Gallivan et al., 1989). A value of 0.9 was used in this study.

Sample collection

Blood was collected from the coccygeal artery using a 22 gauge, 2.54 cm (1") hypodermic needle. The bovine coccygeal artery is a suitable source for blood-gas analysis (Collie, 1991; Nagy et al., 2002). Arterial blood unlike venous blood can fill a heparinized syringe without applying suction. Therefore, minimal, if any, negative pressure was applied to the syringe chamber by drawing on the plunger when obtaining a sample. Approximately, 2 – 2.5 ml of blood was collected in a 3 ml syringe. Syringes were heparinized with approximately 0.25 ml of sodium heparin (1,000 IU/ml). The plunger of each syringe was pulled back to the 3 ml mark coating the inner chamber surface with heparin. Heparin was then expelled so that only the needle hub contained heparin. Collection of blood up to the 2 ml mark results in dilution of the blood sample by < 5 %. Dilution < 10 % is sufficient to minimize pre-analytic error (Hutchison et al., 1983). The sample was discarded if during collection the flow of arterial blood was interrupted. Air bubbles within the blood were immediately expelled and the first several drops of blood discarded before analysis. Blood-gas analysis was performed using a handheld analyser (VetScan i-STAT 1, Abaxis, Union City, CA, USA). Results are automatically stored under the animal identification number. A temperature 'correction' algorithm was used to adjust blood-gas tensions according to rectal temperature (CLSI, 2001).

Ultrasound measurements were made using an Aloka SSD-500V ultrasound console and 3.5 MHz 17 cm linear array transducer (Aloka CO., LTD, Tokyo, Japan). Ultrasonography images were collected between ribs 12 and 13. Backfat thickness and *L. dorsi* cross-sectional area was determined from the analysis of images using Bovine Image Analysis software application (Designer Genes Technologies, Inc., Harrison, AR). Hip height was measured from the ground to the iliac crest.

Statistical Procedures

Statistical analyses were performed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Generalized estimating equations were used to account for the repeated measures (Liang and Zeger, 1986; Zeger and Liang, 1986) as the same calves in herd A were measured on 2 separate occasions. An exchangeable correlation structure was used. Hip height was forced into the model to account for the functional maturity of the cardio-pulmonary system (Lekeux et al., 1984). Herd was included as a fixed effect to account for clustering.

RESULTS

When controlling for hip height calf age was not significantly associated with *l. dorsi* area ($P = 0.40$) and was removed from the model. Herd ($P = 0.74$), sex ($P = 0.07$), hip height ($P < 0.001$) and A-a O_2 pressure gradient ($P = 0.001$) were included in the final model. No significant interactions were present. Bulls had a *l. dorsi* area 3.0 ± 1.4 cm^2 larger than heifers ($P = 0.03$) and 2.9 ± 2.1 cm^2 larger than steers ($P = 0.06$) when controlling for other variables in the model. *L. dorsi* area did not differ between steers and heifers ($P = 0.57$). A 1 cm increase in hip height was associated with a 0.6 ± 0.1 cm^2 increase in *l. dorsi* area ($P < 0.001$) when controlling for other variables in the model. An increase in the A-a O_2 pressure gradient of 1 mmHg was associated with a 0.22 ± 0.07 cm^2 decrease in *l. dorsi* area ($P = 0.001$) (Fig. 1). Median L-lactate levels were high for calves in both herds (Table 2). The mean A-a O_2 pressure gradient for all calves was 23.0 ± 0.9 mmHg and ranged from 2.3 - 43.6 mmHg. This range in A-a O_2 pressure gradients is associated with an absolute difference in *l. dorsi* area of 9.20 ± 2.73 cm^2 when controlling for herd, sex and hip height.

Table 2. Portable analyzer arterial blood values[1]

	Herd A		Herd B
	07/01/11	10/27/11	09/27/11
pH	7.44 ± 0.04	7.45 ± 0.06	7.42 ± 0.09
pCO_2, mmHg	38.3 ± 5.7	38.2 ± 3.3	40.4 ± 8.9
pO_2, mmHg	74.1^{a} ± 12.3	69.6^{ab} ± 13.9	67.1^{b} ± 15.1
Lactate,[2] mmol/L	3.23	1.78	2.89
Hematocrit, %	36.9^{a} ± 2.4	33.8^{b} ± 2.9	36.1^{c} ± 1.9
A-a O_2, mmHg	24.5^{a} ± 7.7	26.4^{a} ± 7.8	18.8^{b} ± 7.4

[1] Mean ± SD provided for normally distributed data.
[2] Median provided due to lack of normal distribution.
Within a row, means without a common superscript differ ($P \leq 0.05$).

DISCUSSION

The efficacy of O_2 transfer from the alveoli into the pulmonary blood can be estimated from the A-a O_2 pressure gradient. An A-a O_2 pressure gradient > 10 mmHg is considered to be abnormally high due to diffusion impairment, right-to-left vascular shunt or more commonly, ventilation-perfusion mismatching (Lekeux, 1993). The high A-a O_2 pressure gradients reported in our study would be considered pathologic in other mammalian species. There are multiple plausible explanations. However, we speculate that the root cause is likely to be the limited cardiopulmonary reserve.

Pulmonary lesions are highly prevalent even in apparently healthy feedlot steers (Wittum et al., 1996). Therefore, variation in A-a O_2 pressure gradients may be partially attributable to subclinical pulmonary pathology. Environmental conditions during neonatal development may also be influential. For example, 'pruning' of the pulmonary vasculature of neonatal beef calves has been reported to occur in response to chronic hypoxia (Reeves and Leathers, 1967). Cattle have a lower density of pulmonary capillaries associated with alveoli than the mammalian average (Epling, 1964). Pulmonary vascular 'pruning' due to neonatal hypoxia may increase ventilation-perfusion mismatching. Calves increase their cardiac output in response to a chronic hypoxic stimulus rather than increasing O_2 carrying capacity of the blood (Hays et al., 1978). High pulmonary flow past gas exchange surfaces may impair O_2 exchange. This is suggested to occur in rapidly growing broiler chickens (Wideman et al., 2007). Intrapulmonary shunting is significantly correlated (r = 0.84) with mean PAP in calves and exceeds 10% at mean PAP ≥ 37 mmHg (Cruz et al., 1979). However, all of these suggested mechanisms do not adequately explain the high A-a O_2 pressure gradients reported at low altitude (Gallivan et al., 1991; Lekeux et al., 1984). Further studies are necessary to explain the physiological basis of high A-a O_2 pressure gradients in cattle.

We have demonstrated the positive relationship between efficacious alveolar-arterial O_2 exchange and *Longissimus dorsi* cross-sectional area. Importantly, A-a O_2 pressure gradients may be genetically determined. Spirometric variables in Belgian Blue calves are genetically determined and have a favorable association with body muscling (Bureau et al., 2001). Low A-a O_2 pressure gradients are characteristic of human populations adapted to life in high altitude environments (Moore et al., 1998) suggesting a genetic influence. Genetic selection of cattle for efficacious alveolar-arterial O_2 uptake may reduce the deleterious effect of pulmonary pathology on calf growth.

IMPLICATIONS

Estimation of A-a O_2 pressure gradients provides a measure of O_2 transfer from the lung to the pulmonary circulation irrespective of the underlying cause of impairment. Efficacy of O_2 transfer from the lung into the pulmonary blood may be a limiting factor for optimal muscle growth in calves. This may be particularly true in high altitude environments such as those associated with the Rocky Mountain range.

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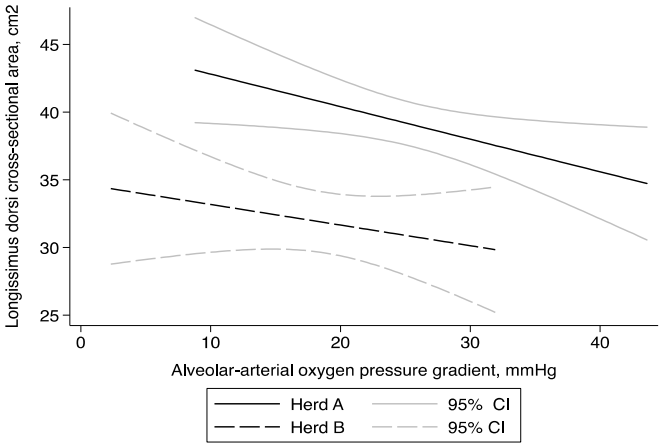

Figure 1. *Longissimus dorsi* cross-sectional area (cm^2) by herd and alveolar-arterial oxygen pressure gradient (mmHg). Regression lines are provided with 95% confidence intervals (95% CI). Calves in herd A and herd B were sampled at 1,466m and 2,008m, respectively.

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THE EFFECTS OF HIGH SULFATE WATER ON GENE EXPRESSION OF MEAT QUALITY IN LAMBS

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ABSTRACT: Livestock water sources with high levels of sulfur (S) or S-compounds are not infrequent in the western and midwestern regions of the U.S. High dietary S can lead to poor performance and health complications in ruminant livestock. In particular, toxic levels of S can lead to polioencephalomalacia (PEM), a neurological disorder characterized by necrosis of the cerebral cortex. Clinical signs of PEM include lethargy, blindness, ataxia, recumbency and seizures. While the effects of S toxicity on ruminant livestock health have been well documented, the effects of a high S diet on meat characteristics are unknown. The objective of this research was to determine the effects of high S drinking water on muscle expression of genes previously associated with carcass quality in meat animals. Wether lambs (n = 20) used for this particular study were assigned one of two treatments: a low-S water (< 100 mg SO_4/L; LS) control treatment or a high-S water (2,400 mg SO_4/L; HS) treatment for 50 d. Latissimus dorsi (LD) and semitendinosus (ST) muscle samples were collected at slaughter (d 51), and RNA was isolated for gene expression analyses. Real-time RT-PCR was used to determine expression levels of insulin-like growth factor (*IGF*) 1, *IGF2*, and calpain. These genes have been implicated in meat quality through roles in protein degradation and meat tenderization. The pH was also determined on all LD and ST muscle samples. The GLM procedure of SAS was used to test for treatment differences and generate least-squares means for gene expression and pH measures. There were no ($P \geq 0.$ 0.234) differences in expression of *IGF1*, *IGF2*, or calpain between LS and HS lambs in either the LD or ST muscle samples. Also, pH did not differ ($P \geq 0.$ 618) between muscle samples of LS and HS lambs. Results from this study indicate that expression of genes potentially important to meat quality is not affected by high levels of S in drinking water in the short term. However, further research is needed to determine the effects of long-term S exposure on other meat quality measures and overall meat production.

Keywords: Gene expression, meat quality, sheep, sulfur

Introduction

Polioencephalomalacia (PEM) is a neurologic disorder of ruminants characterized by necrosis in the cerebral cortex (Gould, 1998). Early disease is characterized by damaged tissue autoflourescing under ultraviolet light. Clinically, PEM is characterized by blindness, ataxia, recumbency, and seizures. Polioencephalomalacia was traditionally thought to be associated with altered thiamine metabolism. However, it has since been determined that PEM is associated with S toxicity, acute lead poisoning, and water deprivation-sodium ion toxicosis (Gould, 1998). Polioencephalomalacia can occur with high-grain diets, which can promote the growth of thiaminase-producing bacteria, diets that include plants high in thiaminases, such as bracken fern, and diets high in S (Purdue University, 2002). In range cattle, the disease tends to occur in late summer and fall, whereas in feedlot cattle and sheep it more typically occurs in the winter. The maximum tolerable dietary concentration for S is 0.4% DM in cattle and sheep; > 0.4% S DM may induce clinical PEM (Gould, 2000). Adequate nutrition is the best way to prevent PEM. However, removing animals from areas of high S and providing palatable low-S feed and water is necessary for prevention.

Insulin-like growth factor-1 (*IGF1*) and insulin-like growth factor-2 (*IGF2*) are hormones that have been associated with muscle hypertrophy (Suzuki et al., 2004) as well as intramuscular fat deposition and meat tenderness (Han et al., 2008). Calpain is a cysteine protease and is considered the chief proteolytic enzyme involved in postmortem degradation of myofibrillar proteins (Underwood, 2007). Because of their roles in meat quality, these genes may serve as indicators of effects on muscle traits or quality. Therefore, the goal of this study was to gain a better understanding of the genetic and homeostatic relationships of elevated dietary S on meat quality in production livestock. The objectives of this research were to assess changes in expression of genes related to meat quality and determine changes in pH of muscle samples collected from sheep exposed to high S water.

Materials and Methods

Animal procedures. All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee. Wether lambs (n = 80) were randomly assigned to one of four treatments (20 wethers per treatment): 1) low-S water (<100 mg SO_4/L; LS); 2) high-S water (2,400 mg SO_4/L; HS); 3) high-S water (2,400 mg SO_4/L) plus a low-Fe supplement (250 ppm $FeCO_3$); or 4) high-S water (2,400 mg SO_4/L) plus a

high-Fe supplement (500 ppm $FeCO_3$) for a 50 d period (Jons et al., 2011). From this study, 20 wether lambs from the LS and HS treatments were randomly selected for further gene expression and pH analyses. The wethers received ad libitum access to a pelleted diet (5.9% soy hulls, 17.5% dried distillers grain solubles, 2.5% molasses, 2% limestone, 1% white salt, 0.5% ammonium chloride, 0.5% trace mineral salt, and 0.1% deccox) and water. Individual feed intake was monitored using a GrowSafe system, and daily water intake of each group was tracked by measuring water disappearance daily. Body weights were recorded on d -1, d 25, and d 50 to estimate ADG. This also allowed for daily feed intake, daily water intake, and ADG to be analyzed in two periods (Period 1: d 0 to d 25; Period 2: d 26 to d 50) to identify any changes in performance associated with early and more chronic treatment effects. Both daily feed and water intake were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for fixed effects of treatment, period, and their interaction as repeated measures using the autoregressive variance/covariance structure, which had the lowest BIC value. Average daily gain was analyzed for fixed effects of treatment, period, and the treatment by period interaction also using the MIXED procedure. Least squares means of all performance traits (daily feed and water intake; ADG) were estimated and tested for pair-wise differences using the Tukey adjustment, assuming an α level of 0.05.

Tissue collection. Wethers were slaughtered at the University of Wyoming abattoir at the end of the trial period. Latissimus dorsi (LD) and semitendinous (ST) muscle samples were collected in triplicate from each animal at slaughter and snap-frozen on dry ice for gene expression analyses and determination of pH. Size of LD and ST were not recorded at slaughter.

RNA extraction. Frozen tissue (50-100 µg) was placed in 1 mL of TRI reagent (Sigma Chemical Co., St. Louis, MO) and homogenized using an electronic tissue grinder (IKA Laboratories; Wilmington, NC) for 30 sec at maximum speed. The homogenate was allowed to sit at room temperature for 5 min and then 0.2 ml of chloroform was added with shaking. The homogenate was incubated at room temperature for another 10 min and then centrifuged for 15 min at 12,000 g and 4°C. The upper aqueous layer was removed and placed in a new tube containing 0.5 ml of isopropanol, mixed and incubated at room temperature for 10 min. The RNA was precipitated by centrifuging for 10 min at 12,000 g and 4°C.

The RNA pellet was resuspended in 100 µL RNase-free water and further purified using the RNeasy kit (Qiagen, Santa Clarita, CA). Briefly, 350 µL buffer RLT (supplied by the kit) was added to each sample followed by 250 µL absolute ethanol. The samples were mixed and pipetted onto RNeasy columns provided in the kit. Samples were centrifuged and the flow-through was discarded. The columns containing RNA were washed with buffer RW1 (supplied by the kit) and centrifuged again. Columns were allowed to incubate for 15 min at room temperature with 80 µL DNase 1 solution (supplied by the kit) followed by another wash with RW1. Columns were washed twice with buffer RPE and the RNA was eluted in 100 µL RNase-free water. The purified RNA was checked for quantity and quality using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Denver, CO) and 2 µg aliquots were placed in 0.5 mL tubes for cDNA synthesis.

cDNA synthesis and Real-Time RT-PCR. Two µg of RNA was mixed with 4 µL reverse transcription buffer (5X) and 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA) in 20 µL total volume. The mixture was placed in a thermocycler for 5 min at 25ºC, 30 min at 42ºC, 5 min at 85ºC, and held at 4ºC. The cDNA was diluted with 100 µL nuclease-free water and stored at -20ºC until real-time RT-PCR was performed. Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) such that amplicons were approximately150 bp in size. Real-time RT-PCR was performed by mixing 10 µL of diluted cDNA with 12.5µL of SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), 500 pmol each forward and reverse primer, and 0.5 µL nuclease-free water in each well of a 96-well plate. Amplification was performed using the IQ5 and 40 cycles of 95ºC for 30 sec and 60ºC for 30 sec. Melting curve analysis was performed post-amplification to ensure the quality of PCR products as noted by the presence of a single peak. Briefly, the PCR plate was heated to 95°C for 3 min, cooled to 55°C, and then the temperature increased by 0.5°C /sec up to 95°C. Ovine glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the reference gene and all gene expression levels were quantified and reported relative to *GAPDH* expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Real-time RT-PCR was performed in duplicate for each sample/primer set. Relative expression values were tested for a treatment effect, assuming an α level of 0.05, and least squares means were estimated using the GLM procedure of SAS.

pH analysis. Both LD and ST muscle samples were individually ground (100 mg/mL in dH_20) for 30 sec at high speed with a 15 sec pause for cool-down time. Each muscle sample was diluted with another 1 mL of water and then analyzed for pH using a standard pH meter. pH levels were tested for treatment effects and least squares means were estimated using the GLM procedure in SAS.

Results and Discussion

Performance measures. As previously reported by Jons et al. (2011), daily feed intake was not affected by the main effect of treatment or by the interaction between treatment and period. However, feed intake was affected (P = 0.006), as expected, by the period effect, with greater daily feed intake in the second period (d 26 to d 50). Similarly, daily water intake was greater (P = 0.006) in the second period, but no effects of treatment or the interaction of treatment and period were determined. Finally, ADG was greater (P = 0.001) in the initial period (d 0 to d 25), but not different across LS and HS treatments. These results suggest that this level of dietary S (2,400 mg SO_4/L) does not noticeably affect lamb performance.

Gene expression. Levels of mRNA for *IGF1* did not differ in LD (P = 0.234) or ST (P = 0.366) muscles of

HS versus LS lambs. Also, mRNA levels of *IGF2* did not differ in LD ($P = 0.941$) or ST ($P = 0.550$) muscles between HS and LS lambs. Finally, *calpain* message in LD ($P = 0.882$) and ST ($P = 0.823$) muscle was similar between HS and LS lambs.

Both *IGF1* and *IGF2* have been associated with fat deposition and meat quality in hogs. Previous studies conducted by Buonomo and Klindt (1993) suggested that plasma concentrations of *IGF1* are positively associated with growth rate and gain:feed, suggesting that *IGF1* plays a role in promoting lean muscle growth. Coleman et al. (1994) further suggested that circulating levels of *IGF1*, originating from liver, muscle, or fat may stimulate lean tissue growth through several endocrine pathways. Suzuki et al. (2004) indicated that *IGF1* was a moderate indicator of growth and fat accumulation in pigs at 8 wk of age. The true role of *IGF1* in relation to carcass and meat quality is still unclear, particularly in ruminants. It has been suggested that high concentrations of *IGF1* are correlated with increased backfat thickness (Bunter et al., 2002); however, Suzuki et al. (2004) and Owens et al. (1999) suggested that *IGF1* controls lean muscle growth, whereas *IGF2* regulates adipose tissue growth.

Insulin-like growth factor-2 has been strongly implicated in the development of adipose tissue growth. A study conducted by Owens et al. (1999) suggested *IGF2* is not related to growth rate or gain:feed ratio, but is positively correlated with backfat thickness. This conclusion was supported by Buonomo and Klindt (1993), who indicated that pigs genetically selected for increased backfat depth consistently had higher plasma *IGF2* levels. Gatford et al. (1996) also implicated *IGF2* in backfat deposition in prepubertal sheep.

Calpain is the foremost proteolytic enzyme involved in postmortem myofibrillar protein degradation (Underwood, 2007). Calpain degrades minor muscle proteins, thus fragmenting the muscle structure. Fragmentation weakens the structure, allowing the meat to be more easily broken down. Research has shown that feeding β-adrenergic agonists to increase lean muscle deposition can increase muscle toughness postmortem. Calpain enzymes are inhibited by β-adrenergic agonists, thus supporting the idea that calpain plays a key role in meat quality and tenderness (Kretchmar et al., 1990). Research conducted by Koohmaraie et al. (1995) also indicated that expression of the callipyge gene in sheep causes specific changes to calpain enzymes resulting in tough meat.

Tissue pH. The pH of LD samples in LS (5.65 ± 0.05) and HS (5.68 ± 0.05) wethers was not different ($P = 0.618$). Additionally, there was no difference ($P = 0.651$) in pH of ST samples in LS (5.74 ± 0.07) versus HS (5.78 ± 0.07) wethers. Meat tenderness and calpain activity are closely linked to pH of the muscle. Calpain activity is optimal at neutral pH. Muscle tenderness is affected by pH changes postmortem. Ultimate shear force and myofibrillar fragmentation assessed postmortem in sheep indicated that muscles with intermediate pH (5.4-6.7) had the highest shear force and lowest fragmentation value (Watanabe et al., 1996). Muscles with the highest pH (6.7-6.4) had the lowest fragmentation value, whereas muscles with the lowest pH had the largest fragmentation value.

Implications

This study indicated that high dietary S had no effect on muscle expression of *IGF1*, *IGF2*, or calpain, nor on muscle pH. However, the level of S administered in this study also did not elicit an appreciable change in performance. Further studies involving increasing concentration and duration of S administration may be warranted to better understand the potential implications of high dietary S, or even S toxicity, on meat quality characteristics of livestock.

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Table 1. Sequence of primers used for real-time RT-PCR.

Gene acronym	Gene name	Forward primer	Reverse primer
IGF1	Insulin-like growth factor	ATGCCATGTCACATCCTCCTC	CTCCAGCCTCCTCAGATCAC
IGF2	Insulin-like growth factor	ACCCTCCAGTTTGTCTGTGG	GGGGTATGCTGTGAAGTCGT
Calpain	Cysteine protease	ACATCTGAGGGCTTTGAGGA	GAAGGTGACAGCCTCCATGT
GAPDH[1]	Glyceraldehyde 3-phosphate-dehydrogenase	GATTGTCAGCAATGCCTCCT	GGTCATAAGTCCCTCCACGA

[1]Reference gene. All gene expression levels were quantified and reported relative to *GAPDH* expression using the $2^{-\Delta\Delta CT}$ method.

Table 2. Real-time RT-PCR expression levels of insulin-like growth factor *(IGF)-1*, *IGF-2*, and calpain genes in low-S and high-S treated wethers.

	Treatment		
	LS[1]	HS[2]	*P*-value[3]
	Latissimus dorsi (LD) muscle		
Insulin-like growth factor-1	3.20 (0.58)	4.18 (0.56)	0.234
Insulin-like growth factor-2	3.29 (0.51)	3.34 (0.49)	0.941
Calpain	1.64 (0.18)	1.68 (0.17)	0.882
	Semitendinous (ST) muscle		
Insulin-like growth factor-1	4.15 (1.12)	5.58 (1.07)	0.366
Insulin-like growth factor-2	11.66 (2.57)	9.49 (2.45)	0.550
Calpain	2.13 (0.19)	2.07 (0.19)	0.823

[1]LS = low-S water (<100 mg SO_4/L) treatment.

[2]HS = high-S water (2,400 mg SO_4/L) treatment.

[3]*P*-value from least squares means comparison; significance assumed at an α level of 0.05.

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EFFECT OF MEAL ALFALFA MIXED (10%) CALVES STARTER ON THE BEHAVIOR OF DAIRY CALVES IN RAISING IN AN ARID REGION OF MEXICO

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ABSTRACT. The objective of this study was to evaluate the effects of level (10 %) of alfalfa meal *(Medicago sativa L.)* mixed in calf starter ration on dairy calves behavior on calf birth weight (CBW), weight at 60 d (W60) and daily gain from birth to 60 d of age (ADG) of Holstein calves in a dairy herd located in a desert region of Baja California, Mexico. Calf starter in the farm facility ICA-UABC. Sixty two dairy calves (n= 35 females and n= 27 males) of two days of age were assigned randomly to one of two groups: group 1 (n=32) fed with calf starter ad libitum; group 2 (n=30) fed with calf starter and alfalfa meal mixed 10 % ad libitum. Calves consumption 2 L of colostrum at 6 and 12 h after birth. In both groups 2 liters of whole milk were given every 12 hours until weaning. Calves starter (14.78 %) and calf starter meal mixed was provided (14.90 % CP) beginning at two days of age. Calf birth weight was recorded as well as weights of calves at 30 d to weaning (60 d of age). Statistical analyses were carried out by GLM procedure (General Linear Models) of the Statistical Analysis System program (SAS Institute Inc.). Average weight of calves at birth (CBW), was 31.501 ± 1.19, and 34.387 ± 1.21 kg, respectively, in females and males. In groups 1 and 2, the weights at 30 d vas 42.847 ± 1.21 and 41.204 ± 1.25kg, respectively, were not different (P > .05). The weights at 60 d was 60.771 ± 1.72 and 58.009 ± 1.80 kg, respectively, in groups 1 and 2, were not different (P > .05). Average daily weight gain (ADG)**,** in groups 1 and 2, at 60 d was 0.427 ± 0.019 and 0.416 ± 0.02 kg, respectively, were not different(P>.05) . These results show that in both study groups, the diet offered can influence behavior in raising calves, although the results did not differ if it is economical to use the calf starter mixed r, although it was not the economic evaluation. The use of alfalfa meal mixed in calf starter (10%) can be ways of initiators include concentrates with low protein as used in this study and be more economical, although the results were not different. Future work is needed to determine the long term effects of forage containing diets.

Key Words: dairy calves, alfalfa meal, desert region, calf starter.

Introduction

The intake of solid feed is vital to the calf for making the transition from a preruminant animal to a functioning ruminant. However, there is still much controversy concerning the composition of starter that should be fed to preruminant calves, especially regarding the level of forage those diets should contain. Forage consumption promotes muscular development of the rumen (Tamate et al., 1962; Hamada et al., 1976) and stimulates rumination and flow of saliva into the rumen (Hodgson, 1971). However, forage digestion by microorganisms does not provide sufficient concentrations of rumen VFA, especially butyrate, required for optimal papillae development. Fermentation of concentrates provides the necessary butyrate to stimulate papillae development, but these feeds may promote keratinization of the papillae in calves and lambs (Thompson et al., 1958; Nocek et al., 1980).

The amount of roughage necessary in the diet of young calves is unclear (Klein et al., 1987). Trials investigating the use of forage in starter rations have yielded inconsistent results. Several investigators concluded that forage addition to the diet increases starter intake (Kincaid, 1980; Thomas and Hinks, 1982; Stobo et al., 1985). However, others have seen a negative impact of forage addition on the consumption of starter rations (Hibbs et al., 1956; Whitaker et al., 1957; Leibholz, 1975).

Ration particle size influences the ruminal environment, volatile fatty acid production, and papillae structure and function. Diets that are chopped or ground to fine particle sizes decrease rumen pH and cellulolytic bacteria populations (Beharka et al., 1998). This decrease in pH is caused by a lack of rumination and saliva flow into the rumen in calves and mature cows (Kellaway et al., 1977; Santini et al., 1983). Ruminal papillae of animals receiving small forage particles have increased keratinization (McGavin and Morrill, 1976a). This decrease in active tissue results in decreased VFA absorption (Hinders and Owen, 1965; Nocek et al., 1980; Greenwood et al., 1997b). Papillae begin to branch to compensate for the loss in metabolically active tissue (McGavin and Morrill, 1976b; Greenwood et al., 1997b; Beharka et al., 1998).

Determining the proper level of forage, if any, to include in starter diets for optimal rumen development will benefit the producer greatly by shortening the length of time the calf requires milk replacer and allow early weaning of a mature ruminant calf. There are several advantages of early weaning when compared to the prolonged feeding of milk replacer. Labor is greatly reduced when calves are fed starter rations when compared to feeding milk replacer. Calves that are weaned earlier have fewer digestive problems (Klein et al.,1987). Determining the optimal level of forage necessary to develop the rumen properly will allow producers to wean younger calves that have more mature digestive systems. This should prevent the decline in intake and growth seen immediately after weaning. The objective of these studies was to determine the effect of

form of diet and the inclusion of various levels of controlled particle size of hay on rumen development, growth, and health parameters in restricted-fed and ad libitum fed calves.

Materials and Methods

The study was conducted in the Experimental Unit Cattle Milk Institute of Agricultural Sciences of the UABC, located in the Ejido Nuevo Leon, BC, 42 km SE of Mexicali, BC. 62 Holstein calves were used (n = 35 females and n = 35 males) who were born in August 2010 to March 2011). Calves both females and males were distributed at random to the following treatments, begining at two days of age: Group 1 (n = 19 females and n = 13 males) consumed calves starter (14.8% CP) ad libitum; Group 2 (n = 16 females and n = 14 males) consumed calves starter (14.90% CP), which contained 10% ground alfalfa (meal) mixed ad libitum. All calves after birth were separated from mothers and housed in individual pen in raising room, where they were given 2 L of colostrum at 6 and 12 hours old, were later provided 2 L of whole milk every 12 hours until weaning (60 d of age). We recorded the weight of calves at birth, 30 and 60 d of age to estimate their weight and daily gain (ADG). GLM procedure (General Linear Models) of the Statistical Analysis System program (SAS Institute Inc, 2002).

Results and Discussion

Table 2 shows the mean and standard deviation of the results on the birth weights of calves (n = 62), females (n = 35 females) and males (n = 27) in both study groups. The females had lower weight (P <.05) than males, which were 31.501 ± 1.195 and 34.387 ± 1.210 kg, respectively. These results are not new and there is generally a strong effect on the sex of calves, since males are heavier at birth than females.

The weight at 30 and 60 d of age (weaning), are presented in Table 3. The weight at 30 d in both study groups did not differ (P> 0.05), resulting in the following averages, 42.8475 ± 1.214 and 41.2048 ± 1.256 kg in calves that consumed the calves starter and calves starter mixed with 10% alfalfa meal, respectively. The weight at 60 d showed no difference (P> 0.05) in the groups in this study, with the following averages: 60.771 ± 1.724 and 58.009 ± 1.805 kg in Group 1 and 2, respectively. Coverdale et al. (2004), averages were lower (W60) when initiator included in concentrate 7.5 and 15% bromegrass hay ground that the initiator concentrate. However Greenwood et al. (1997b) weights were 15% greater when included bromegrass hay ground mixed in concentrate initiator. Also Beharka et al. (1998), including 25% alfalfa ground initiator concentrate had higher weaning weights (W60). The findings of this study show a positive trend for the weight at 30 and 60 d of age with respect to Group 1, but showed no statistical difference, suggesting an analysis of economic feasibility so that it can recommend to producers.

Table 4 shows the mean of the ADG a 30-60 d, did not differ (P> .05) in the groups under study, being this of 0.5767± 0.300 and 0.554 ± 0.031 kg in group 1 and 2 respectively. A similar pattern occurred in the ADG of 0-60 d, which was 0.427 ± 0.019 and 0.416 ± 0.020 kg in group 1 and 2, respectively. Coverdale et al. (2004) obtained ADG averages lower than those found in this study, when included in concentrate initiator 7.5 and 15% bromegrass hay ground that the initiator concentration. Just as in body weight, ADG also has a slight positive trend towards the group consuming only the initiator concentration, so further research is needed that level will be adequate forage.

Conclusions

This study shows that both the incorporation of forage and the particle size can influence the intake and behavior of calves. Forage of a consistent particle size (ground) can be used successfully in raising initiation rations. The addition of alfalfa meal at concentrate initiator may be a way to reduce feed costs in rearing calves, although in this study we make in economic analysis. Further work is needed to determine the level and type of forage, to see the effects in starter diets in dairy calves.

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Table 1. Composition of the diets on a dry basis in group 1 and 2.

Measurment	Group 1 Calves Starter	Group 2 Calves Starter (10% alfalfa meal)	Alfalfa Meal
Dry Matter	92.69	92.77	89.56
Humidity	7.31	7.23	10.44
Crude Protein	14.78	14.90	18.91
Cenizas	4.22	4.69	10.83
Ether Extract	1.26	1.35	1.44
Neutral Detergent Fiber (NDF)	13.29	14.56	44.04
Acid Detergent Fiber (ADF)	5.72	7.74	35.93

Nutrition Laboratory Animal of ICA-UABC

Table 2. Mean and standard deviation of body weight (kg) at birth of the animals under study.

Sex of calves	Number of Animals	Mean ± SD
Females	35	31.501 ± 1.195^{a}
Males	27	34.387 ± 1.210^{b}
Total Animals	62	

[a,b] Different letters indicate significant difference (P<.05).

Table 3. Mean and standard deviation of body weight (kg) at 30 and 60 days of age of the dairy calves under study.

Treatment	Number of Animals	30 d	60 d
		Mean ± SD	Mean ± SD
Group 1	32 (f=19 and m=13)	42.847 ± 1.214^{a}	60.771 ± 1.724^{a}
Group 2	30 (f=16 and m=14)	41.204 ± 1.256^{a}	58.009 ± 1.805^{a}
Total Animales	62		

[a] Equal letters in columns indicate no significant difference (P>.05).

Table 4. Mean and standard deviation of the average daily gain (ADG) to 30-60 and 0-60 days of age the animals under study.

Treatment	Number of Animals	30 - 60 d	0 - 60 d
		Mean ± SD	Mean ± SD
T1	32 (f=19 and m=13)	0.5767 ± 0.300^{a}	0.4275 ± 0.019^{a}
T2	30 (f=16 and m=14)	0.5547 ± 0.031^{a}	0.4168 ± 0.020^{a}
Total Animals	62	62	62

[a] Equal letters in columns indicate no significant difference (P>.05).

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GLUCOSE TOLERANCE AND GROWTH CHARACTERISTICS OF YEARLING BEEF HEIFERS FROM DIFFERENT NUTRITIONALLY DEVELOPED ANGUS GRANDSIRES.

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ABSTRACT: Twenty-six yearling Angus heifers grandsired by two different nutritionally developed bulls were compared based on glucose tolerance, gain, and carcass merit. Grandsires included a forage-based (FORAGE) and a grain-based (GRAIN) Angus bull. Performance was based on a 92 d restricted growing period (LEG), a 69 d grazing period (GRZ), and a 95 d feedlot period (FDLT). Intravenous glucose tolerance tests (IVGTT) were conducted at initiation of the LEG, GRZ, and FDLT periods. Heifers were exposed to similar nutritional management during each period. Shrunk BW and carcass ultrasound measurements were collected prior to each IVGTT and carcass data were obtained at slaughter. Means were analyzed as a completely randomized design using heifer within grandsire as the error term and heifer sire as a covariate. Pearson correlations were determined on IVGTT area under the curve (AUC) estimates against all performance measures. No differences ($P > 0.10$) were detected for ADG, backfat (BF), or LM depth during the LEG period, though GRAIN heifers showed greater ($P = 0.024$) marbling deposition. Glucose AUC_{LEG} was not different ($P > 0.10$) between grandsires and only tended ($P = 0.065$) to be correlated (r = 0.44) with LEG BF deposition. No differences ($P > 0.10$) were detected for ADG, ultrasound measures, or glucose AUC across grandsires during the GRZ period. Glucose AUC_{GRZ} was inversely correlated (r = -0.495; $P = 0.019$) with ADG_{GRZ} across grandsires, with a stronger association with FORAGE (r = -0.68; $P = 0.015$) vs. GRAIN (r = -0.45; $P = 0.19$) heifers. The GRAIN heifers had a greater ($P < 0.05$) ADG_{FDLT} and quality grade, and tended ($P = 0.058$) to have a greater marbling score. Glucose AUC_{FDLT} was not different ($P = 0.12$) between grandsires, but tended ($P < 0.15$) to be correlated with marbling score (r = +0.45) and quality grade (r = +0.46) of GRAIN heifers. Influence of grandsire nutrition on heifer performance was minimal during the pre-feedlot period, but affected feedlot ADG and carcass quality. Glucose tolerance measures were not definitively associated with most measures of growth and carcass merit.
Keywords: glucose tolerance, beef heifers

Introduction

As cost of most feedstuffs have increased over the past decade or so, there has been growing interest in marketing and purchasing beef sires that have been developed in forage-based programs without supplemental grain. These forage-based sires have been touted as 'more efficient' converters of forage to growth versus conventional sires that have been developed in "grain-based" management programs. When evaluating beef systems the evaluation of selected sires should include both terminal and retained offspring to determine long-term impacts of sire selection on production outcomes. In a commercial crossbreeding program the influence of replacement heifer sire (grandsire) is diminished in their offspring are bred to alternate sires. Therefore, the heritable effects of certain production efficiencies, such as forage gain efficiencies, are difficult to quantify. The current study utilized intravenous glucose tolerance testing (**IVGTT**), along with ADG and carcass merit, to evaluate the potential heritable influences of different nutritionally-developed grandsires on yearling feeder heifer performance.

Materials and Methods

All animal procedures were approved by the Oregon State University Institute of Animal Care and Use committee (ACUP#4305). Twenty-six Angus-cross yearling heifers (261 ± 5.1 kg; 365 ± 3.8 days of age) grandsired by either a forage-developed Angus bull (**FORAGE**) or a grain-developed Angus bull (**GRAIN**) were evaluated using IVGTT and growth performance during low-energy growing (**LEG**), pasture grazing (**GRZ**), and feedlot finishing (**FDLT**) periods, along with carcass merit. Heifers were sired by either a single AI Angus sire (n = 20) or a single natural Angus sire (n = 6), with each sire represented in each grandsire category.

Initially, heifers were stratified by grandsire and housed in one of six drylot pens. Heifers were removed from feed for 24 h to obtain shrunk BW, carcass ultrasound, and IVGTT measurements prior to the LEG period. During the LEG period, heifers received a low-energy diet (DM basis: 0.68 Mcal NEg/kg, 11.8% CP, and 2.5% lipid) once per day for 92 d. Interim LEG gain performance was determined every 30 d after trial initiation based on 24 h shrunk BW. Duration of LEG period was based on spring grass production, and was terminated once grass accumulation could sustain grazing pressure. Shrunk BW, carcass ultrasound, and IVGTT measurements were collected prior to start of GRZ period.

Heifers were commingled in a common pasture (mixed cool-season grasses) during the GRZ period for 69 d. Heifers had ad libitum access to fresh water and mineral. The GRZ period was concluded once overall grass production was not able to maintain at least 50% of the available grazing DM per heifer, based on visual assessment. At conclusion of the GRZ period, heifers were withdrawn from feed for 24 h after which BW, carcass ultrasound, and IVGTT measurements were collected.

Heifers were transported 195 km to a commercial feedlot facility for finishing. Heifers were managed in a common pen and received a finishing diet (DM basis: 1.46 Mcal NEg/kg, 12.5% CP, and 7.55% lipid) and anabolic implant (Finaplix-H; Intervet Inc., Millsboro, DE). Heifers were slaughtered at a commercial abattoir when subcutaneous fat accumulation reached approximately 1.27 cm, based on visual estimation by trained feedlot personnel. Carcass measurements were obtained by trained OSU personnel 23 h post-mortem.

Intravenous glucose tolerance testing. The IVGTT procedures used were based on modifications of Wastney et al. (1982) and Mir et al. (1998). Briefly, following a 24 h fasting period, heifers were restrained and two blood samples (baseline glucose values) were obtained via jugular venipuncture 5 min apart. A disposable 64 mm intravenous catheter (Terumo Corp., Somerset, NJ) was then inserted into the right jugular vein followed immediately by a 50% dextrose solution dosage of 0.3 g glucose/kg shrunk BW. Blood samples were collected at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min post-infusion via catheter extension into 4 ml evacuated tubes containing sodium fluoride and potassium oxalate (Vacuette; Greiner Bio-One North America Inc., Monroe, NC). Following each collection approximately 1 to 2 ml of 2.9% sodium citrate was infused to maintain catheter patency. Prior to each collection, approximately 2 to 3 ml of blood was evacuated using a syringe and discarded. Blood samples were then placed on ice until centrifugation (2000 × *g* for 30 min at 4°C). Plasma was harvested and stored at -20°C until analysis. Plasma glucose concentrations were determined using a commercial enzymatic hexokinase colorimetric assay (Glucose Liqui-UV., Proc. 1060; Stanbio Laboratories, Boerne, TX). Area under the curve (**AUC**) for glucose concentrations were calculated using the Net Increment method of the Trapezoidal Rule (Shiang, K., 2004), with the pre-dosing glucose concentrations considered the baseline.

Ultrasound measurements. Carcass ultrasound measurements were obtained prior to IVGTT measurements during each collection period. Measurements for intramuscular fat or marbling (**UMARB**), longissimus muscle depth (**UMD**), and subcutaneous fat or backfat (**UBF**) were obtained at the 12th to 13th-rib interface by an experienced technician. Ultrasound images were generated using an Aloka 500V (Aloka Co., Ltd, Wallingford, CT) B-mode instrument equipped with a 3.5-MHz, 125 mm general purpose transducer array (UST-5011U-3.5). Images were collected by a single technician with software from the Cattle Performance Enhancement Company (CPEC, Oakley, KS). Estimates of UBF, UMD, and UMARB were based on image analysis programming (Brethour, 1994) contained within the CPEC software program.

Statistical analysis. Means were analyzed as a completely randomized design using heifer within grandsire as the error term and heifer sire as a covariate (Cochran and Cox, 1992). Pearson correlation coefficients were determined on glucose AUC estimates against all performance and carcass measures. Statistical models were conducted using MIXED and REG procedures of SAS (SAS Institute Inc., Cary, NC). Probabilities with α-levels less than 0.05 were considered significant, while α-levels greater than 0.05 and less than or equal to 0.15 were considered statistical tendencies.

Results and Discussion

No differences ($P > 0.15$) were reported for initial BW, UBF, UMD, or UMARB between grandsire heifer groups (Table 1). Gain performance was similar ($P > 0.15$) across grandsire heifer groups, along with changes in UBF and UMD during the LEG period (Table 1). The GRAIN heifers accumulated greater ($P = 0.024$) amounts of marbling according to UMARB estimates during the LEG period. The ADG observed during the LEG period was greater than predicted by NRC (2000), which indicates the diet may not have been as low in digestible energy as initially predicted. Regarding gain AUC_{LEG} tended ($P < 0.15$) to be positively correlated with FORAGE heifers for ADG, whereas it tended ($P < 0.15$) to be positively correlated with backfat and marbling accumulation in GRAIN heifers (Table 2).

During the GRZ period (Table 1), both grandsire heifer groups gained at a similar rate ($P = 0.62$) and accumulated similar ($P > 0.15$) amounts of muscling and intramuscular fat according to UMD and UMARB estimates, respectively. The ultrasound data indicated that FORAGE heifers tended ($P = 0.15$) to accumulate more backfat during the GRZ period compared to the GRAIN heifers. Glucose AUC_{GRZ} (Table 2) was negatively (r = -0.680) associated with ADG in FORAGE heifers ($P = 0.015$), but not for GRAIN heifers ($P = 0.19$). Though numerically AUC_{GRZ} was inversely associated with all ultrasound measures across grandsires, none were statistically significant ($P > 0.15$; Table 2).

The GRAIN heifers had a 15% greater ($P = 0.040$) ADG during the FDLT period, though final BW and HCW were not different ($P > 0.15$) between grandsire heifer groups (Table 1). Carcass backfat, ribeye area, KPH, and yield grade were similar ($P > 0.15$) across grandsire groups, but GRAIN heifers tended to have greater marbling score ($P = 0.058$) and subsequent quality grade ($P = 0.044$). Final retail yield was similar ($P = 0.56$) between grandsire heifer groups. Glucose AUC_{FDLT} (Table 2) tended ($P = 0.13$) to be positively associated (r = +0.487) with ribeye area in FORAGE heifers, whereas it tended to be positively associated with marbling score (r = +0.454; $P = 0.14$) and subsequent quality grade (r = +0.460; $P = 0.13$) in GRAIN heifers. Interestingly, AUC_{LEG} of GRAIN heifers was positively associated (r = +0.637; $P = 0.065$) with final yield grade, while FORAGE heifers showed an equally negative association (r = -0.645; $P = 0.061$) with final yield grade (Table 2). Though many of the correlations were not significant, both AUC_{LEG} and AUC_{GRZ} of GRAIN heifers indicated positive associations with most feedlot and carcass variables, while FORAGE heifers had negative associations.

Implications

The influence of grandsire nutritional development was minimal in growing and finishing heifers, with the greatest influence associated with feedlot ADG and carcass marbling and quality grade. Glucose tolerance testing was varied in regards to grandsire influence and production variables, but potentially indicates differences in glucose utilization between grandsire heifer sources during both growing and finishing periods.

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Table 1. Gain, ultrasound, glucose tolerance, and carcass merit measures of yearling heifers grandsired by either forage- or grain-developed bulls.

Item	GRAIN[1]	FORAGE[1]	SEM	*P*-value
Trial initiation				
BW, kg[2]	256.2	262.6	7.79	0.57
UBF, mm[3]	3.61	3.35	0.182	0.34
UMD, mm[3]	43.09	44.76	1.084	0.30
UMARB[3, 13]	4.33	4.34	0.025	0.62
Glucose AUC[4]	12976	11982	975.4	0.50
End of growing (LEG) period				
ADG, kg/d[5]	1.01	1.07	0.030	0.21
Δ UBF, mm[3, 6]	+1.30	+1.12	0.287	0.67
Δ UMD, mm[3, 6]	+9.09	+10.35	1.701	0.62
Δ UMARB[3, 6]	+0.72	+0.29	0.115	0.024
Glucose AUC[4]	11756	10578	531.2	0.14
End of grazing (GRZ) period				
ADG, kg/d[5]	0.76	0.72	0.045	0.62
Δ UBF, mm[3, 6]	+0.53	+1.26	0.341	0.15
Δ UMD, mm[3, 6]	+4.00	+3.08	1.594	0.69
Δ UMARB[3, 6]	-0.24	-0.24	0.115	0.99
Glucose AUC[4]	10149	8790	588.1	0.12
End of feedlot (FDLT) finishing period				
Final BW, kg[7]	576	561	9.87	0.29
ADG, kg/d[5]	1.83	1.59	0.076	0.040
HCW, kg	394	384	6.75	0.29
Backfat, cm	1.35	1.46	0.085	0.38
Ribeye area, cm[2]	91.1	89.6	2.14	0.62
KPH, %	2.23	2.15	0.143	0.53
Yield grade[8]	2.80	2.86	0.138	0.79
Marbling score[9]	621	567	18.7	0.058
Quality grade[10]	740	721	6.3	0.044
Retail yield, %[11]	54.64	54.35	0.338	0.56

[1]Grandsires nutritionally-developed on forage (FORAGE) or grain (GRAIN).
[2]Shrunk BW
[3]Initial ultrasound measurement estimates of backfat (UBF), LD depth (UMD), and marbling score (UMARB).
[4]Area under the curve (AUC) calculated from intravenous glucose tolerance tests.
[5]Based on shrunk BW.
[6]Change (Δ) in ultrasound measurement estimates from beginning to end of period.
[7]Calculated using carcass weights divided by average dressing percentage.
[8]Yield grade = 2.5 + (2.5 × backfat) + (0.0038 × HCW) + (0.2 × KPH) – (0.32 × ribeye area).
[9]Marbling score: 400 = slight, 500 = small, 600 = modest, 700 = moderate.
[10]Quality grade: 500 = select, 600 = choice, 700 = prime.
[11]Retail yield = 51.34 – (5.78 × backfat) – (0.0093 × HCW) – (0.462 × KPH) + (0.740 × ribeye area).

Table 2. Correlation coefficients of glucose tolerance testing with growing (LEG), grazing (GRZ), and feedlot finishing (FDLT) periods of yearling heifers grandsired by either forage- or grain-developed bulls.

		AUC_{LEG}[1]		AUC_{GRZ}[1]		AUC_{FDLT}[1]	
Item		r =	*P*-value	r =	*P*-value	r =	*P*-value
Growing (LEG) period							
$UBF_{initial}$[2]	GRAIN	0.023	0.95				
	FORAGE	0.200	0.61				
$UMD_{initial}$[2]	GRAIN	0.410	0.27				
	FORAGE	0.330	0.39				
$UMARB_{initial}$[2]	GRAIN	0.334	0.38				
	FORAGE	0.068	0.86				
ADG	GRAIN	0.221	0.57	0.98	0.79		
	FORAGE	0.517	0.15	-0.117	0.72		
$\Delta UBF^{2,3}$	GRAIN	0.523	0.15	0.005	0.99		
	FORAGE	0.156	0.69	-0.266	0.40		
$\Delta UMD^{2,3}$	GRAIN	0.134	0.73	-0.251	0.48		
	FORAGE	-0.294	0.44	0.107	0.74		
$\Delta UMARB^{2,3}$	GRAIN	0.540	0.13	0.344	0.33		
	FORAGE	-0.364	0.34	-0.378	0.23		
Grazing (GRZ) period							
ADG	GRAIN	-0.34	0.37	-0.453	0.19	-0.468	0.12
	FORAGE	-0.181	0.64	-0.680	0.015	-0.326	0.33
$\Delta UBF^{2,3}$	GRAIN	-0.389	0.30	0.001	0.99	-0.243	0.45
	FORAGE	-0.006	0.99	-0.148	0.65	-0.512	0.11
$\Delta UMD^{2,3}$	GRAIN	-0.555	0.12	-0.190	0.60	-0.363	0.25
	FORAGE	0.222	0.57	-0.180	0.58	-0.007	0.98
$\Delta UMARB^{2,3}$	GRAIN	-0.178	0.65	-0.477	0.16	0.067	0.84
	FORAGE	0.294	0.44	-0.005	0.99	0.300	0.37
Feedlot (FDLT) finishing period and carcass characteristics							
ADG	GRAIN	0.192	0.62	0.504	0.14	0.229	0.47
	FORAGE	-0.693	0.038	-0.296	0.35	-0.313	0.35
HCW	GRAIN	0.112	0.77	0.275	0.44	0.257	0.42
	FORAGE	-0.226	0.56	-0.026	0.94	0.054	0.87
Backfat	GRAIN	0.413	0.27	0.453	0.19	0.341	0.28
	FORAGE	-0.250	0.52	-0.127	0.69	0.105	0.76
Ribeye area	GRAIN	-0.689	0.040	0.236	0.51	0.051	0.88
	FORAGE	0.297	0.44	0.228	0.48	0.487	0.13
KPH	GRAIN	-0.106	0.79	0.100	0.78	0.192	0.55
	FORAGE	-0.306	0.42	-0.471	0.12	-0.246	0.47
Marbling score	GRAIN	0.141	0.72	0.137	0.71	0.454	0.14
	FORAGE	-0.409	0.27	-0.502	0.096	-0.017	0.96
Quality grade	GRAIN	0.131	0.74	0.167	0.64	0.460	0.13
	FORAGE	-0.371	0.33	-0.486	0.11	0.020	0.95
Yield grade	GRAIN	0.637	0.065	0.133	0.71	0.254	0.42
	FORAGE	-0.645	0.061	-0.395	0.20	-0.284	0.40

[1]Area under the curve (AUC) calculated from intravenous glucose tolerance tests performed at beginning of growing (LEG), grazing (GRZ), and finishing (FDLT) periods.

[2]Initial ultrasound measurement estimates of backfat (UBF), LD depth (UMD), and marbling score (UMARB).

[3]Change (Δ) in ultrasound measurement estimates from beginning to end of the period.

PASTURES AND FORAGES

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PROTEIN SUPPLEMENTATION OF LOW-QUALITY COOL-SEASON AND WARM-SEASON FORAGES

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ABSTRACT: An in situ study (Exp. 1) was performed using four ruminally cannulated steers (473 ± 13 kg BW) in a completely randomized design to compare the in situ degradation parameters of 2 low-quality, cool-season (C3) forages (Meadow foxtail, 4.5% CP; Reed canarygrass, 2.6% CP) and a warm-season (C4) forage (tallgrass prairie, 5.1% CP). Incubation time points were 0, 2, 8, 12, 24, 48, and 96 h. Means were separated using protected LSD ($P \leq 0.05$). Also, a digestion study (Exp. 2) utilizing 6 ruminally cannulated steers (428 ± 12 kg BW) in a 6 × 5 incomplete Latin square design was conducted to compare CP supplementation of low-quality C3 and C4 forages. Treatments included Meadow foxtail (MF; 4.6% CP), Reed canarygrass (RC; 2.6% CP) and tallgrass prairie (TG; 5.2% CP) hays with and without supplemental CP. Experimental periods were 20 d and soybean meal (50.2% CP) was used as the source of supplemental CP. Hays were offered at 120% of the previous 5 d average intake. No differences in lag time ($P = 0.83$) or rate of disappearance ($P = 0.58$) were noted for NDF in Exp. 1. However, NDF effective degradability was greatest for MF (49%; $P \leq 0.05$) while TG (45%; $P \leq 0.05$) was greater than RC (36%). Rate of N disappearance was similar for MF and RC ($P > 0.05$) with both C3 forages greater than TG ($P \leq 0.05$). Also, RDP as a proportion of total CP was greatest in MF (62%; $P \leq 0.05$) with RC (54%) greater than TG (41%; $P \leq 0.05$). However, effective degradability of N was greatest for MF (79%; $P \leq 0.05$) and for TG (64%; $P \leq 0.05$) compared with RC (58%). In Exp. 2, hay and total DMI was increased with supplementation ($P < 0.01$), for C3 compared with C4 ($P < 0.01$) and for MF compared with both RC ($P < 0.01$) and TG ($P < 0.05$). Also, apparent total tract DM digestibility was increased with supplementation ($P = 0.03$) while not affected by forage type ($P = 0.34$) but was greater for MF compared with both RC ($P < 0.01$) and TG ($P = 0.02$). These data indicate that low-quality C3 forages have greater RDP and N degradation rates than low-quality C4 forages. In addition, our data suggests that MF is better forage than RC and TG for beef cattle based on increased DMI and digestibility.

Key words: cattle, cool-season, forage, protein, supplementation, warm season

Introduction

Beef cattle in the Intermountain West normally consume low-quality cool-season (C3) 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). However, research suggests that CP supplementation of ruminants consuming low-quality C3 forages does not increase forage DMI in a manner similar to that observed with warm-season (C4) forages (Mathis et al., 2000; Bohnert et al., 2002; 2011). Therefore, the objective of this experiment was to compare DMI, digestibility, and ruminal fermentation of ruminants offered low-quality C4 (tall grass-prairie hay) and C3 hays (Meadow foxtail and Reed canarygrass) with and without supplemental CP in the hope of elucidating the reason(s) for the apparent difference in forage intake response.

Materials and Methods

All experimental procedures used in this study were approved by the Oregon State University Institutional Animal Care and Use Committee (ACUP# 4256).

Experiment 1. In Situ Degradation of C3 and a C4 low-quality forages

Four ruminally cannulated Angus × Hereford steers (473 ± 13 kg BW) were used in a completely randomized design to evaluate the ruminal degradation characteristics of 2 low-quality, C3 forages (Meadow foxtail; MF; Reed canarygrass; RC) and a C4 forage (tallgrass prairie; TG; Table 1). Steers had ad libitum access to low-quality meadow hay (6.5% CP; DM basis). A full description of meadow hay has been provided by Wenick et al. (2008). Steers were offered the low-quality meadow hay diet for at least 90 d prior to the start of Exp. 1.

Dacron bags (10 × 20 cm; Ankom Technology Corp., Fairport, NY) were labeled with a waterproof permanent marker, weighed, and 4 g (air equilibrated) of ground (2-mm; Wiley Mill; Model 4; Arthur H. Thomas, Philadelphia, PA) forage were added and the bags sealed with an impulse sealer (TISH-200; TEW Electric Heating Equipment CO., LTD, Taipei, Taiwan). Triplicate bags for each forage source were placed in a bucket containing warm water (39°C) and introduced into the rumen within 5 min. Bags were placed in a weighted polyester mesh bag within the rumen of each steer (0, 2, 8, 12, 24, 48, and 96 h) in reverse order, allowing all bags to be removed simultaneously. Three blank Dacron bags were incubated for 96 h and used to correct for microbial and feed contamination. Upon removal, Dacron bags were rinsed under tap water until the effluent was clear and dried at 55°C for 24 h. The dried triplicates were allowed to air equilibrate for 24 h at room temperature, weighed for residual DM, composited by steer, time and forage type,

and analyzed for NDF (Robertson and Van Soest, 1981) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). The NDF residue was then weighed and analyzed for N (Leco CN-2000; Leco Corp., St. Joseph, MI).

Kinetic variables for DM, NDF, and N digestibility were estimated with SAS (SAS Inst., Inc., Cary NC) using the modified nonlinear regression procedure described by Fadel (2004). Effective degradability of NDF and N was determined as described by Hoffman et al. (1993) using a ruminal passage rate of 2%/h (Mass et al., 1999). Rumen degradable protein (**RDP**) was calculated as described by Ørskov and McDonald (1979) with rumen undegradable protein (**RUP**) calculated as 1- RDP. Data were analyzed using the MIXED procedure of SAS. The model included forage source as the independent variable. Steer was used as random variable. Means were separated using LSD protected by a significant F-test ($P \leq 0.05$).

Experiment 2. Forage intake and nutrient digestibility of C3 and a C4 low-quality forages with and without supplemental CP

Six ruminally cannulated steers (428 ± 12 kg BW) were used in an incomplete 6 × 5 Latin square design (Cochran and Cox, 1957) and housed in individual pens (4 x 4 m) within an enclosed barn with continuous lighting. Steers were provided continuous access to fresh water and the 2 low-quality C3 forages and the single C4 forage used in Experiment 1; 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 3 × 2 factorial design (3 forages with or without supplemental CP). Soybean meal (**SBM**) was placed directly into the rumen via the ruminal cannula for supplemented treatments. The amount of CP supplied by SBM was 0.11% of BW/d. The supplemented treatments were formulated, based on preliminary forage and SBM samples, to provide approximately 100% of the estimated DIP requirement assuming a microbial efficiency of 10%.

Experimental periods were 20 d, with intake measured beginning d 13 and concluding d 18. On d 14, 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 and SBM were collected d 13 through d18 and orts were collected on d 14 through 19. Forages, SBM, and orts were dried at 55°C for 48 h and ground in a Wiley mill (1-mm screen). On d 15 through 20, 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 (-20°C), dried at 55°C for 96 h, and 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 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).

Data were analyzed as an incomplete 6 × 5 Latin square using the MIXED procedure of SAS and Satterwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The model included treatment and period as independent variables. Steer was used as random variable. Contrasts used were: 1) supplemented vs not supplemented; 2) C3 vs C4; 3) contrast 1 × contrast 2; 4) meadow foxtail vs reed canarygrass; 5) meadow foxtail vs tall grass prairie.

Results and Discussion

We had a wider range in forage CP than anticipated for the hays used in Experiments 1 and 2, with the greatest values occurring with TG (5.1 and 5.2%, respectively) and the least occurring with RC (2.6 in both experiments; Table 1).

Experiment 1

The A fraction (total pool disappearing at a rate to rapid to measure) of NDF was greater for MF compared with RC and TG ($P \leq 0.05$) with no difference noted between RC and TG ($P > 0.05$; Table 2). The B fraction (degradable fraction disappearing at a measurable rate) was comparable between MF and TG ($P > 0.05$) while the proportion of degradable NDF in RC was approximately 22% less than TG and MF ($P \leq 0.05$). The undegradable fraction of NDF (C fraction) was approximately 70% greater ($P \leq 0.05$) for RC compared with TG and MF. Also, though no differences ($P = 0.58$) were noted in the rate of NDF degradation, the effective degradability of NDF was greatest ($P \leq 0.05$) for MF (48.7%) followed by TG (45.2%) which was more digestible than RC (35.6%; $P \leq 0.05$).

The A fraction of N was greatest with RC ($P \leq 0.05$) followed by MF. Also, both C3 forages had a greater A fraction than TG ($P \leq 0.05$; Table 2). However, when evaluating the B fraction, MF and TG were similar in ruminal degradable N ($P > 0.05$) but greater than RC ($P \leq 0.05$). Consequently, the undegradable N fraction was greatest for RC ($P \leq 0.05$) while TG was greater than MF ($P \leq 0.05$). The rate of N degradation was similar for the C3 forages ($P > 0.05$) which were almost 75% greater than that observed with the C4 forage ($P \leq 0.05$). This agrees with work by Bohnert et al. (2011) in which the N degradation rate of TG was almost 70% less than that observed with Kentucky bluegrass straw (C3; *Poa pratensis*). The proportion of RDP, as well as the effective degradability of N, was greatest for MF ($P \leq 0.05$). Also, RC contained a greater proportion of RDP than TG ($P \leq 0.05$). This agrees with a results reported by Bohnert et al. (2011) in which a low-quality C3 forage had approximately 28% greater RDP than a C4 forage with comparable CP concentration. The effective degradability of N was greater for TG compared with RC ($P \leq 0.05$).

Experiment 2

Hay and total DMI were increased with supplementation ($P < 0.01$; Table 3) and were greater for the C3 forages compared with the C4 ($P < 0.01$). These results agree with Bohnert et al. (2011) who reported similar results when comparing CP supplementation of TG with Kentucky bluegrass straw. However, in contrast to Bohnert et al. (2011), we did not note a supplementation × forage type interaction for hay intake ($P = 0.65$). Also, MF had greater hay and total DMI than RC ($P < 0.01$) or TG ($P < 0.01$). The differences between the current study and Bohnert et al. (2011) are probably due to differing nutritional quality profiles of the forages and/or the different C3 forage species used.

Apparent GIT digestibility of DM and N was increased with supplementation ($P \leq 0.03$; Table 3) but not affected by forage type ($P \geq .34$). However, digestibility of DM and N was greater with MF compared to RC ($P < 0.01$) and digestibility of DM was greater ($P = 0.02$) and N digestibility tended to be greater ($P = 0.06$) for MF compared with TG.

Ruminal IADF fill was not influenced by supplementation ($P = 0.11$; Table 3) but was decreased for MF compared to RC ($P < 0.01$) and tended to be less compared with TG ($P = 0.08$). However, supplementation increased both ruminal passage rate ($P < 0.01$) and outflow rate ($P < 0.01$) of IADF with no influence of forage type ($P \geq 0.80$) or for MF compared with RC ($P \geq 0.21$) and TG ($P \geq 0.38$).

Implications

The data reported here adds to the growing body of evidence that intake of low-quality C3 forages by ruminants is greater than intake of C4 forages. However, it is not evident what specific nutritional quality factors are causing the increased forage intake with C3 compared with C4. Consequently, further research is warranted to help ascertain the indices that will assist nutritionists to better predict forage intake of ruminants consuming low-quality forages. Also, our data indicates that MF is better forage than RC and TG for beef cattle based on increased DMI and digestibility.

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Table 1. Feedstuff[a] nutrient content (DM basis)

Nutrient,%	MF	RC	TG	SBM
Exp. 1				
CP	4.6	2.6	5.1	--
NDF	63.6	68.9	77.7	--
Exp. 2				
CP	4.6	2.6	5.2	50.2
NDF	64.1	69.0	77.1	16.1
ADF	33.6	41.5	42.0	5.6
IADF	21.3	32.8	28.6	0.0

[a] MF = Meadow foxtail hay (cool-season forage); RC = Reed Canarygrass hay (cool-season forage); TG = tallgrass prairie hay (warm-season forage); SBM = soybean meal.

Table 2. Ruminal degradation parameters of two cool-season forages (meadow foxtail and reed canarygrass) and one warm season forage (tallgrass prairie).

Degradation Parameters	Meadow Foxtail	Reed Canarygrass	Tallgrass Prairie	SEM[a]	P-Value
NDF					
Fractions, %[b]					
A	7.21^{x}	4.51^{y}	4.74^{y}	0.41	0.006
B	68.7^{x}	55.0^{y}	72.1^{x}	3.5	0.03
C	24.2^{x}	40.6^{y}	23.1^{x}	3.29	0.02
Kd[c], /h	0.031	0.028	0.026	0.0043	0.58
Effective Degradability, %[d]	48.7^{x}	35.6^{y}	45.2^{z}	0.98	< 0.001
N					
Fractions, %					
A	28.2^{x}	36.4^{y}	11.7^{z}	0.53	< 0 .001
B	51.1^{x}	21.8^{y}	52.2^{x}	1.41	< 0.001
C	20.7^{x}	41.9^{y}	36.2^{z}	1.23	< 0.001
Kd[c], /h	.0613^{x}	.0738^{x}	.0388^{y}	0.0078	0.02
RDP[e], % of CP	62.4^{x}	54.2^{y}	41.4^{z}	1.03	< 0.001
RUP[f]	37.6^{x}	45.8^{y}	58.6^{z}	1.03	< 0.001
Effective Degradability, %[d]	79.3^{x}	58.1^{y}	63.8^{z}	1.23	< 0.001

[a] n = 4.
[b] A = soluble fraction (total pool disappearing at a rate too rapid to measure); B = degradable fraction (total pool disappearing at a measurable rate); C = undegradable fraction (total pool unavailable in the rumen).
[c] Fractional rate constant.
[d] Calculated as A + {B × [(*K*d/(*K*d + *K*p)]}, where *K*p was the ruminal passage rate, which was set at 2%/h (Hoffman et al., 1993). The units used for *K*d in the equation were per hour.
[e] Calculated as described by Ørskov and McDonald (1979).
[f] Calculated as 1 − RDP.
[x,y,z] Means in a row without a common superscript are different (P < 0.05).

Table 3. Intake, digestibility, and ruminal IADF dynamics by beef steers consuming low-quality cool-season (C3; Meadow foxtail; Reed canarygrass) and warm-season (C4; tallgrass prairie) grass hays with or without soybean meal (CP) supplementation

								P-Value[b]				
Item	MF	MF+	RC	RC+	TG	TG+	SEM[a]	Con vs Supp.	C3 vs C4	Supp. × Type	MF vs RC	MF vs TG
Intake, g/kg BW												
Hay DMI	17.4	22.5	14.8	20.0	14.6	19.2	0.724	< 0.001	0.004	0.65	< 0.001	< 0.001
Supplement DMI	0.00	1.09	0.00	1.09	0.00	1.09						
Total DMI	17.4	23.6	14.8	21.1	4.6	20.3	0.72	< 0.001	0.004	0.65	< 0.001	< 0.001
N	0.127	0.256	0.066	0.176	0.121	0.247	0.0061	< 0.001	< 0.001	0.44	< 0.001	0.17
NDF	11.3	14.8	10.4	14.1	11.5	15.2	0.572	< 0.001	0.13	0.92	0.16	0.52
IADF	4.0	5.0	4.3	5.8	3.8	5.2	0.46	0.002	0.52	0.83	0.21	0.94
Apparent GIT Digestibility, %												
DM	50.0	59.4	37.7	45.1	41.7	47.7	4.23	0.03	0.34	0.73	0.004	0.02
N	26.8	51.8	-22.5	33.2	11.4	36.9	7.86	< 0.001	0.79	0.27	< 0.001	0.06
NDF	46.2	56.6	36.6	36.9	47.9	48.6	6.80	0.50	0.48	0.70	0.04	0.65
ADF	36.8	45.0	26.7	29.1	38.1	37.0	8.71	0.66	0.68	0.68	0.15	0.70
Ruminal IADF												
Fill, g/kg BW	8.9	8.3	11.2	10.0	10.2	9.4	0.67	0.11	0.73	0.93	0.006	0.08
Passage rate, %/h	1.82	2.63	1.64	2.49	1.63	2.34	0.243	0.002	0.49	0.80	0.55	0.38
Outflow, (g/kg BW)/h	0.165	0.208	0.178	0.240	0.158	0.217	0.0192	0.002	0.52	0.83	0.21	0.94

[a] n = 5.

[b] Con vs Supp. = non-supplemented vs supplemented treatments; C3 vs C4 = cool-season vs warm-season forages; Supp. × Type = interaction of supplementation and forage type; MF vs RC = Meadow foxtail vs Reed canarygrass; MF vs TG = Meadow foxtail vs tallgrass prairie.

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HIGH-TANNIN FORAGE UTILIZATION BY BEEF COWS IV. EFFECTS OF CORN STEEP LIQUOR SUPPLEMENTATION ON PERFORMANCE AND HERBIVORY PATTERNS OF BEEF COWS GRAZING NATIVE RANGE INFESTED BY SERICEA LESPEDEZA (*LESPEDEZA CUNEATA*)

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ABSTRACT: Sericea lespedeza (**SL**) is classified as a noxious weed in Kansas. We reported previously that supplementation with corn steep liquor (**CSL**) increased acceptance of and tolerance for SL-contaminated hay by beef cows fed in confinement. Therefore, the objective of this trial was to determine if supplemental CSL would promote herbivory of actively-growing SL by beef cattle grazing native rangeland in the Kansas Flint Hills. Herbivory of SL and performance of lactating, crossbred cows and calves (n = 145; initial cow BW = 579 ± 91 kg) were evaluated during a 5-mo experiment. Native tallgrass pastures (n = 9; 50 ± 17 ha) infested heavily by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cows or grazing by cows supplemented with CSL (1.79 kg DM ∗ cow^{-1} ∗ d^{-1}; 45% DM, 34% CP). Cow-calf pairs were assigned randomly to treatment and pasture (stocking rate = 0.5 ha/AUM). Animal BW were measured monthly from June 1 to October 1; cows were assessed for BCS at those times also. Total forage biomass and SL biomass were measured monthly. Herbivory of SL was estimated along line transects in October. Cow BW change, BCS change, and pregnancy rates were not different ($P \geq 0.22$) and calf ADG was not different ($P \geq 0.29$) between treatments. Additionally, total forage biomass and SL biomass were not different ($P = 0.88$) between treatments; however, both varied ($P < 0.01$) by month of the grazing season. The percentage of individual SL plants showing evidence of herbivory tended to be greater ($P = 0.09$) on pastures grazed by supplemented cows than on pastures grazed by unsupplemented cows (94 vs. 80% of SL stems, respectively). Under the conditions of our study, beef cows supplemented with CSL appeared to put greater grazing pressure on SL than unsupplemented beef cows; however, animal performance was not influenced by CSL supplementation.

Keywords: beef cows, corn steep liquor, *Lespedeza cuneata*

Introduction

Sericea lespedeza (*Lespedeza cuneata*, **SL**) is classified as an invasive plant throughout the Great Plains. It infests approximately 2,530 km^2 of grassland in Kansas (Wang et al., 2008; USDA, 2010). The aggressive nature of the plant reduces native grass production on a site-specific basis by up to 92%, through a combination of prolific seed production, canopy dominance, and allelopathy (Kalburtji and Mosjidis 1992; Dudley and Fick, 2003; Eddy et al. 2003; Vermeire et al., 2007). Herbicides retard the spread of SL but application is expensive; moreover, herbicides are lethal to ecologically-important, non-target plant species (Eddy et al., 2003). Goat grazing reduced SL seed production significantly (Mayo, 2000; Hart, 2001); however, widespread use of goat grazing faces significant cultural and economic challenges in the Kansas Flint Hills (Pacheco et al., 2012).

Increased grazing pressure on SL by beef cattle, the most economically-relevant herbivore in the region, may slow its spread and facilitate some measure of biological control. Unfortunately, mature plants contain high levels of condensed tannins which reduce protein digestion by beef cattle and are a strong deterrent to grazing (Mantz et al., 2009).

Supplemental corn steep liquor (**CSL**) alleviated the negative effects of condensed-tannin ingestion by beef cattle fed prairie hay contaminated with SL (Eckerle et al., 2011b). In addition, beef cows supplemented with CSL did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial (Eckerle et al., 2011c). Therefore, the objective of our study was to evaluate the effects of supplemental CSL on herbivory patterns and performance of beef cows grazing native tallgrass rangeland infested by SL.

Materials and Methods

Animal care practices used in our study were approved by the Kansas State University Animal Care and Use Committee (protocol no. 2978).

Location. Our study was conducted between May 1 and October 1, 2011 in Chautauqua County, KS on 9 native tallgrass pastures located approximately 16 km southeast of Sedan, KS. Pastures were burned April 10. Plant-species composition of pastures was estimated immediately before initiation of the trial using a modified step-point technique (Table 1; Owensby, 1973).

Table 1. Botanical composition of native tallgrass pastures grazed from May 1 to October 1

Item		Percent
Grasses		**83.22**
Big bluestem	*Andropogon gerardii*	19.50
Little bluestem	*Schizachyrium scoparium*	16.94
Sedges	*Carex* spp.	14.11
Indiangrass	*Sorghastrum nutans*	7.88
Scribner's panicum	*Dichanthelium oligosanthes*	5.00
Tall dropseed	*Sporobolus asper*	4.94
Switchgrass	*Panicum virgatum*	2.44
Sand paspalum	*Paspalum setaceum*	2.17
Green bristlegrass	*Setaria geniculata*	1.89
Hairy grama	*Bouteloua hirsuta*	1.67
Purple top	*Tridens flavus*	1.33
Sideoats grama	*Bouteloua curtipendula*	0.50
Blue grama	*Bouteloua gracilis*	0.17
Other grasses	*n = 21*	4.68
Forbs		**14.29**
Lance-leaf ragweed	*Ambrosia bidentata*	2.38
Western ragweed	*Ambrosia psilostachya*	1.42
Grassleaf goldenrod	*Euthamia graminifolia*	1.28
Sericea lespedeza	*Lespedeza cuneata*	0.96
Heath aster	*Symphyotrichum ericoides*	0.59
Purple prairie clover	*Dalea purpurea*	0.03
Dotted gayfeather	*Liatris punctata*	*TR*
Other forbs	*n = 67*	7.63
Woody plants		**2.49**

Weather. Climatic data were collected from a weather center near Sedan, KS (ID: USC00147305). Monthly average minimum and maximum temperatures and total monthly precipitation were contrasted against the 30-y average for this location (Table 2).

Animals. Lactating, crossbred beef cows with calves (n = 145; initial cow BW = 579 ± 91 kg; initial calf BW = 139 ± 32 kg) were blocked by age and calving date and assigned randomly to 1 of 2 treatments: no supplementation or supplementation with CSL. Cow and calf BW were measured at monthly intervals from June 1 (d 0) to October 1 (d 121); cow BCS (scale = 1 to 9, 1 = emaciated, 9 = obese; Wagner et al., 1988) were assessed also at those times. Cow-calf pairs were allowed to graze freely from May 1 to October 1. Cows were exposed to natural-service breeding from May 1 to July 15. Calves were weaned September 1 at an approximate average age of 200 d (d 93). Cow pregnancy rates were determined via rectal palpation 75 d after bull exposure was terminated (d 121).

Treatments. Native tallgrass pastures (n = 9; 50 ± 17 ha) infested heavily by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cow-calf pairs or grazing by cow-calf pairs supplemented with CSL. Animals were assigned randomly to pastures within designated treatment groups. All pastures were stocked at 0.5 ha/AUM, a rate typical of the Kansas Flint Hills. Beginning June 1, cow-calf pairs were fed supplemental CSL that was delivered 3× / wk in portable feed bunks (61 cm bunk space / cow). Delivery of CSL was prorated for an average daily intake of 3.0 L / cow daily (i.e., 1.79 kg DM / cow daily). Prior research reported that 1.8 kg CSL / cow daily (DM basis) provided complete relief from the symptoms of condensed tannin consumption among beef cows fed SL-contaminated prairie hay (Eckerle et al., 2011b).

Corn steep liquor, a viscous, liquid byproduct of wet-corn milling, was purchased from Archer Daniels Midland in Columbus, NE; each truckload was sampled randomly to determine chemical composition (Table 3).

Herbivory. Two permanent 100-m transects were established in each pasture at the outset of the trial (June 1) in order to estimate above-ground forage biomass, plant-species composition, and SL herbivory. Total forage biomass and SL biomass were estimated by clipping all live plant material from within randomly-placed sampling frames (0.25 m^2; n = 10 / pasture) at a height of 1 cm on 6/1, 7/1, 8/1, 9/1, and 10/1. Forage samples were hand sorted to separate SL from all other forage plants; samples were then placed in separate paper bags to be sun-dried at the collection site for 8h. Because of the remote location of our research site, sun drying was used to prevent aerobic degradation and nutrient loss in forage samples during transport to analytical facilities.

Herbivory of individual SL plants was estimated visually at the end of the study (October 1) at 5-m intervals along each transect. The closest SL plant to each point was examined for evidence of defoliation (e.g., stripped leaves and truncated stems). Plants were scored as either 0 (no evidence of defoliation) or 1 (defoliated).

Chemical composition. Forage, CSL, and SL samples were dried in a forced air-oven (96 h, 50 °C), weighed, and ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen. Dried, ground forage and SL samples were composited across pastures within sampling period and analyzed for DM (16 h, 105 °C), OM (8 h, 450 °C), and N (FP-528, LECO, St. Joseph, Michigan). These samples were also analyzed for NDF and ADF using procedures described by Van Soest et al. (1991; Table 3).

Statistical Analysis. Cow and calf performance were analyzed separately for each period (i.e., early season, late season, and total season) as a mixed model with a one-way treatment structure in a completely-randomized design with subsampling (PROC MIXED; SAS Inst. Inc., Cary, NC). Pasture was the experimental unit and animal was the subsample unit. Class factors included animal, pasture, and treatment. The model statement included only the treatment fixed effect. The random statement had a term for pasture within treatment. Treatment means with standard error and treatment F-test *P*-values were reported for each period.

Cow pregnancy was analyzed as a generalized linear mixed model with a 1-way treatment structure in a completely-randomized design (PROC GLIMMIX; SAS

Inst. Inc., Cary, NC), using the binary distribution with logit-link function. Class factors included pasture and treatment. The model statement included only the treatment fixed effect. The random statement included only the effect for pasture within treatment. Treatment means with SE and F-test *P*-values were reported.

Forage and SL biomass were analyzed in two ways. First, to examine trends in biomass over time, data were analyzed as a mixed model with a 1-way treatment structure in a completely-randomized design with a split-plot in time (PROC MIXED; SAS Inst. Inc., Cary, NC). Class factors included pasture, treatment, and period. The model statement included terms for the fixed effects of treatment, collection date, and treatment × collection date. The random statement included only the effect for pasture within treatment. Treatment × collection-date effects were not detected; therefore, orthogonal polynomial contrasts were used to characterize the biomass trends for collection-date main effect means up through order 4 (i.e., quartic). Second, data were analyzed separately for each collection date, analogous to the analysis for animal performance data, as a one-way treatment structure in a completely-randomized design. The class factor was treatment and no random statement was used. Treatment means with standard error and treatment F-test *P*-values were reported for each period.

The proportion of grazed SL stems was analyzed as a generalized linear mixed model with a 1-way treatment structure in a completely-randomized design (PROC GLIMMIX; SAS Inst. Inc., Cary, NC), using the binomial distribution with logit-link function. Class factors included pasture and treatment. The model included a term for treatment only. The random statement contained terms for pasture within treatment and transect within pasture and treatment. Treatment means with standard error and treatment F-test *P*-values were reported.

For all analyses, means were considered different when $P \leq 0.05$. Tendencies were discussed when $0.05 < P \leq 0.10$.

Results and Discussion

Weather. Weather patterns in 2011 were characterized by below-normal precipitation and above-normal temperatures during the period of our study (Table 2). In general, there was an increase in mean daily high temperature of 0 to 5 °C from May through October; mean daily low temperatures were 6 to 10 °C greater than normal during the same period. Total precipitation in 2011 was approximately 21 cm less than the average observed from 1981 to 2010, whereas precipitation during the period of our study was approximately 60% of normal. The extent to which these abnormally warm, dry conditions influenced the results of our study is not fully known; however, Donnelly (1959) indicated that condensed tannins in SL increased under both above-normal temperatures and below-normal rainfall.

Nutrient Composition. Forage quality followed an anticipated pattern from June to October (Table 3); however, it was generally greater than what has been previously reported for annually-burned, native tallgrass prairie during the summer months (Rao, 1972; Umoh, 1977). Van Soest (1994) indicated that greater-than-expected forage quality was typical of moderate drought, due to abnormally-slow rates of plant maturation. Forage CP concentrations were relatively high during May and October and were least during August, following the hottest, driest month of the year. Concentrations of NDF and ADF were generally the inverse of CP. Interestingly, CP and NDF concentrations in SL were generally more favorable than the average of available pasture forage on a month-by-month basis.

Herbivory. Initial, average, and final total forage biomass and SL biomass were not different ($P \geq 0.52$) between treatments (Table 4). As expected, CSL supplementation did not have an immediate, pasture-scale influence on SL biomass availability. Total forage biomass changed quartically ($P = 0.05$) over time, with forage availability peaking on 7/1 and 10/1 and reaching a nadir on 9/1 (Figure 1). Biomass of SL changed similarly (cubic effect - $P < 0.01$) over time. Biomass of SL was 37% of total forage biomass when averaged over all collection periods.

The proportion of SL plants with visual evidence of herbivory tended to be greater ($P = 0.09$) on pastures grazed by supplemented cows (94.2%) compared with pastures grazed by unsupplemented cows (80.2%; Table 4). Eckerle et al. (2010) established that whole-plant condensed tannins in fresh SL ranged from 16 to 23 % of plant DM during the growing season; moreover, SL condensed tannins were a strong deterrent to DMI (Eckerle et al., 2011a) and greatly reduced ruminal protein digestion (Eckerle et al., 2011b).

Prior research noted that supplementation with CSL appeared to mask or eliminate the post-ingestive consequences of condensed-tannin consumption. Supplemental CSL fed at a rate of 0.6 kg DM daily alleviated the negative effects of condensed tannins on DMI by beef cattle fed prairie hay contaminated with SL; total-tract CP digestion and digestible DMI were normalized when CSL was fed at 1.2 to 1.8 kg DM daily (Eckerle et al., 2011b). Beef cows supplemented with CSL at 0.6 kg DM/d did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial (Eckerle et al., 2011c).

Cow and Calf Performance. Initial cow BW, cow BCS, and calf BW were not different ($P \geq 0.76$) between treatments (Table 5). Supplementation with CSL had no detectable influence ($P \geq 0.22$) on cow BW change, cow BCS change, or calf ADG. Final cow pregnancy rates were also not different ($P = 0.99$) between the treatments. Reasons for lack of response to CSL supplementation on cow and calf performance measures were not immediately clear. We speculated that, under the hot, dry conditions of our study, CSL-supplemented cows may have substituted consumption of supplement for consumption of pasture forage. Alternatively, increased consumption of condensed tannins in SL may have limited performance of CSL-supplemented beef cows.

Cost Analysis. The cost of the CSL at the initiation of our trial was $55.13 / metric ton; cost per cow was estimated at $26.40 for the 120-d period of our study (i.e., 4 kg CSL × 120 d × $0.055/kg; as-fed basis).

A liquid feed-handling system and portable feed bunks (3 × 0.3 m) were purchased to store and feed the CSL at an installed cost of $6,000. Assuming a 5-y period of

depreciation, the annualized cash cost of this equipment was $1,200.

For a 100-hd cow herd, cost for the storage system and bunks would have been $12.00 / cow annually. Delivery of CSL 3× weekly (i.e., 16 wk × 3 deliveries / wk @ $20.00 / delivery) was estimated at $9.60 / cow annually.

Under these conditions, supplementation with CSL cost an estimated $48.00 / cow annually. A commonly-used stocking rate across the Flint Hills of Kansas is 3.25 ha / cow during a 6-month summer grazing season; therefore, the cost of treating an SL infestation using CSL supplementation was estimated at $14.77 / ha annually (i.e., $48.00 ÷ 3.25 ha). Treating SL with herbicides cost an estimated $30 to 40 / ha annually, at the time of this writing. It remains to be seen whether grazing SL with CSL-supplemented beef cattle will provide a degree of control comparable to that achieved with annual herbicide treatment.

Implications

Supplemental CSL fed at 1.79 kg DM / cow daily was associated with increased herbivory of SL during a summer grazing season; moreover, beef cow and calf performance were not negatively affected by condensed-tannin consumption under these circumstances. As expected, CSL supplementation did not have an immediate, pasture-scale influence on SL biomass availability; however, we speculated that repeated applications of CSL supplementation on SL-infested tallgrass pastures may impair seed-producing capabilities of SL.

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Table 2. Growing season weather summary for Chautauqua county, KS (1981 to 2011) [a]

Item	April	May	June	July	August	September	October	Annual
2011								
Average high, °C	22.4	23.9	33.0	38.3	35.9	28.4	24.4	21.7
Average low, °C	15.1	18.1	26.6	30.9	28.7	20.5	15.7	14.7
Precipitation, cm	7.4	10.7	8.1	2.8	10.9	7.7	0.6	85.5
1981 - 2010 mean								
Average high, °C	20.8	25.1	29.6	33.1	33.3	28.4	21.9	20.7
Average low, °C	7.1	12.9	18.0	20.7	19.9	14.6	7.9	7.7
Precipitation, cm	10.0	16.8	14.8	8.7	7.7	10.4	10.4	106.3

[a] Location near Sedan, KS (ID: USC00147305)

Table 3. Nutrient composition of range forage, sericea lespedeza, and corn steep liquor available to beef cows and calves grazing native tallgrass pastures (DM basis).

Item	% DM	% OM	% CP	% NDF	% ADF	% Ca	% P
Range forage							
June 1	92.0	92.6	9.1	53.1	37.2	0.91	0.11
July 1	91.7	93.0	9.2	47.3	36.5	1.08	0.08
August 1	91.8	93.0	7.3	53.5	39.0	0.95	0.08
September 1	91.9	93.3	9.9	46.3	36.3	1.02	0.10
October 1	92.1	93.7	11.1	45.4	38.5	1.09	0.12
SEM	0.05	0.10	0.03	0.55	0.56	0.024	0.005
Sericea lespedeza							
June 1	91.5	93.4	13.1	39.2	33.9	1.19	0.12
July 1	91.5	94.0	11.0	41.7	39.5	1.19	0.08
August 1	91.8	94.3	11.3	43.1	38.4	1.07	0.12
September 1	91.8	94.5	10.6	40.5	41.2	1.23	0.10
October 1	91.8	93.9	13.0	37.1	36.6	1.23	0.13
SEM	0.05	0.02	0.04	0.38	0.58	0.029	0.003
Corn steep liquor	45.1	88.1	34.4	3.1	2.0	0.11	1.90

Table 4. Effects of corn steep liquor supplementation on range forage biomass, sericea lespedeza biomass, and sericea lespedeza herbivory by beef cows and calves grazing native tallgrass pastures.

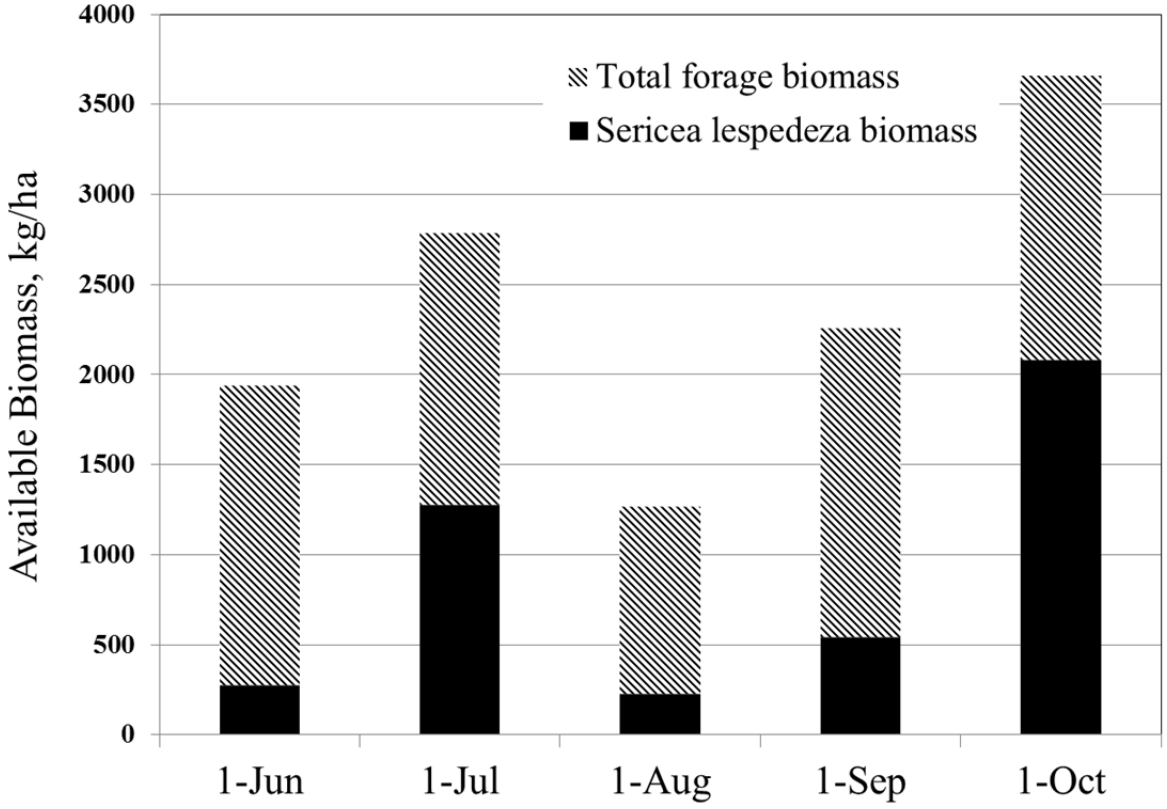
Item	Unsupplemented	Supplemented	SE	*P*
Initial total forage biomass, kg DM/ha	1852	2019	809.3	0.87
Average total forage biomass, kg DM/ha	2312	2445	866.5	0.88
Final total forage biomass, kg DM/ha	3309	4014	809.3	0.52
Initial sericea lespedeza biomass, kg DM/ha	231	310	567.7	0.92
Average sericea lespedeza biomass, kg DM/ha	703	1048	552.7	0.55
Final sericea lespedeza biomass, kg DM/ha	1939	2214	567.7	0.72
Sericea lespedeza stems grazed, % of total	80.2	94.2	6.65	0.09

Table 5. Effects of corn steep liquor supplementation on performance of beef cows and calves grazing native tallgrass pastures.

Item	Unsupplemented	Supplemented	SEM	*P*
Cow BW, kg				
d 0	582	577	15.2	0.84
d 60	609	614	11.3	0.77
d 121	616	629	10.4	0.36
Cow BW change, kg				
d 0-60	27.6	32.3	11.18	0.76
d 60-121	6.4	16.1	5.34	0.22
d 0-121	34.0	49.3	12.51	0.39
Cow BCS [a]				
d 0	5.3	5.2	0.18	0.76
d 60	5.4	5.4	0.11	0.84
d 121	5.0	5.1	0.05	0.27
Cow BCS change				
d 0-60	0.13	0.19	0.223	0.86
d 60-121	-0.41	-0.31	0.103	0.49
d 0-121	-0.28	-0.14	0.164	0.56
Cow pregnancy, % [b]	81.9	82.0	0.05	0.99
Calf BW, kg				
d 0 BW, kg	140	139	6.4	0.93
d 60 BW, kg	215	214	6.9	0.91
d 93 BW, kg	242	244	6.3	0.84
Calf ADG, kg				
d 0-60	1.23	1.24	0.052	0.91
d 60-93	0.81	0.92	0.072	0.29
d 0-93	1.09	1.12	0.033	0.52

[a] Scale = 1 to 9; 1 = emaciated, 9 = obese (Wagner et al., 1988).
[b] Pregnancy was determined via rectal palpation approximately 75 d after removal of bulls.

Figure 1. Total forage biomass [a] and sericea lespedeza biomass [b] on native range grazed by beef cows in the Kansas Flint Hills.



[a] Total forage biomass = sericea lespedeza biomass (black portion of each bar) + biomass from all other forage plants (cross-hatched portion of each bar); quartic effect of time ($P < 0.05$).
[b] Sericea lespedeza biomass = sericea lespedeza biomass as a portion of total biomass; cubic effect of time ($P < 0.01$).

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HIGH-TANNIN FORAGE UTILIZATION BY BEEF COWS V. EFFECTS OF CORN STEEP LIQUOR SUPPLEMENTATION ON DIETARY BOTANICAL COMPOSITION OF BEEF COWS GRAZING NATIVE RANGE INFESTED BY SERICEA LESPEDEZA (*LESPEDEZA CUNEATA*)

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ABSTRACT: Our objective was to determine the effects of supplemental corn steep liquor (**CSL**) on voluntary selection of sericea lespedeza (**SL**) by beef cows grazing native tallgrass range. Dietary botanical composition of cows (n = 145; initial cow BW = 579 ± 91 kg) was evaluated during a 150-d grazing period (5/1 to 10/1). Native pastures (n = 9; 50 ± 17 ha) infested by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cows or grazing by cows supplemented with CSL (1.79 kg DM/cow/d; 45% DM, 34% CP). Cows were assigned randomly to treatment and pasture (stocking rate = 0.5 ha/AUM). Concentration and protein-binding capacity of condensed tannins (**CT**) in SL were measured monthly. Fecal samples were collected from each cow on 6/1, 7/1, 8/1, 9/1, and 10/1. Plant fragments in fecal samples were quantified via a microhistological technique; fragment prevalence in fecal material was assumed to equal botanical composition of the diet. Concentration and protein-binding capacity of CT in SL were greatest ($P < 0.01$) on 8/1 and 9/1, respectively. Prevalence of all graminoids in beef cow diets declined ($P < 0.01$) as the grazing season advanced. Conversely, prevalence of all forbs increased ($P < 0.01$) over time. Prevalence of SL in beef cow diets was influenced ($P < 0.01$) by CSL supplementation and by month. Prevalence of SL in beef cow diets was not different ($P \geq 0.55$) between treatments when concentration and protein-binding capacity of CT were relatively low (6/1, 7/1, and 10/1). In contrast, supplemented cows selected more ($P < 0.01$) SL than unsupplemented cows when concentration and protein-binding capacity of CT were greatest (8/1 and 9/1). We interpreted these data to suggest that voluntary selection of SL by beef cows was inversely related to CT; moreover, supplemental CSL stimulated voluntary selection of SL during periods of high CT. Supplemental CSL did not influence selection of other plant species that were monitored.

Keywords: condensed tannins, diet selection, *Lespedeza cuneata*

Introduction

Over 2,500 km^2 of grasslands in Kansas are infested by the noxious weed, sericea lespedeza (*Lespedeza cuneata*, **SL**; USDA, 2010). Herbicide treatment of SL is expensive; moreover, grassland acreage affected by SL increased over 60-fold between 1988 and 2000, in spite of routine herbicide usage during that period (Eddy et al., 2003).

Nutrient composition of SL appears favorable for livestock production (Sidhu, 2010). Conversely, elevated condensed-tannin content strongly deters voluntary consumption of SL by beef cattle (Eckerle et al., 2011a and 2011b). Increased grazing pressure on SL by beef cattle may slow its spread and facilitate a measure of biological control.

Feedstuffs with tannin-binding properties may promote voluntary consumption of SL by beef cattle. Confined beef steers fed polyethylene glycol (PEG) ate more SL than steers not fed PEG (Mantz et al., 2009); however, use of PEG as an anti-tannin feedstuff is cost-prohibitive and disallowed from a regulatory standpoint in the U.S. (AAFCO, 2008). Eckerle et al. (2011b) reported that moderate amounts of supplemental corn steep liquor (**CSL**; 0.6 to 1.8 kg/d) normalized DMI and total-tract CP digestion by beef cows fed SL-contaminated prairie hay in confinement. Additionally, beef cows supplemented with CSL did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial (Eckerle et al., 2011c). As an inexpensive and palatable byproduct of wet-corn milling, CSL is *Generally Regarded as Safe* by the U.S. Food and Drug Administration (FDA, 2013).

Preedy et al. (2013) indicated that a greater percentage of SL plants were defoliated in pastures grazed by CSL-supplemented cows than in pastures grazed by unsupplemented cows; however, it was unknown if defoliation was related directly to grazing activity of cows. Therefore, our objective was to evaluate the effects of supplemental CSL on botanical composition of the diets of beef cows grazing native tallgrass rangeland infested by SL in the Kansas Flint Hills.

Materials and Methods

Animal care and handling practices used in our study were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 2978).

Location. Our study was conducted between May 1 and October 1, 2011 in Chautauqua County, KS on 9 native tallgrass pastures located approximately 16 km southeast of Sedan, KS. Pastures were burned April 10. Plant-species composition of pastures was estimated immediately before initiation of the trial using a modified step-point technique (Table 1; Owensby, 1973) and presented in a previous publication (Preedy et al., 2013).

Treatments. Nine pastures (50 ± 17 ha) infested heavily by SL (average SL biomass = 37% of total forage biomass) were assigned randomly to 1 of 2 treatments: grazing by unsupplemented cow-calf pairs or grazing by cow-calf pairs supplemented with CSL (45% DM, 34.4% CP). All pastures were stocked at 0.5 ha/AUM, a rate typical of the Kansas Flint Hills. Cow-calf pairs were fed supplemental CSL that was delivered 3×/wk in portable feed bunks (61 cm bunk space/cow) beginning on June 1; CSL was fed to achieve an average intake of 3.0 $L*cow^{-1}*day^{-1}$ (i.e., 1.79 kg DM / cow daily). Eckerle et al. (2011b) reported that 1.8 kg $CSL*cow^{-1}*day^{-1}$ (DM basis) relieved symptoms of condensed-tannin consumption among beef cows fed SL-contaminated prairie hay. Chemical composition of CSL (Archer Daniels Midland, Columbus, NE) was reported previously (Preedy et al., 2013).

Animals. Lactating, crossbred beef cows with calves (n = 145; initial cow BW = 579 ± 91 kg) were blocked by age and calving date and assigned randomly to treatments (no supplementation or supplementation with CSL) and to pastures. Cow-calf pairs were allowed to graze assigned pastures freely from May 1 to October 1. Cows were exposed to natural-service breeding from May 1 to July 15. Calves were weaned September 1 at an approximate age of 200 d. Cow pregnancy rates were determined via rectal palpation 75 d after bull exposure was terminated. Cattle performance data were reported previously (Preedy et al., 2013). Beef cows were gathered on 6/1, 7/1, 8/1, 9/1, and 10/1, individually restrained in a squeeze chute (~ 2 min), and fresh fecal-grab samples were collected from each animal. Each grab sample was hand-mixed to ensure homogeneity and a 40-g subsample was retained for analysis. Grab samples were sealed in plastic containers, immediately placed on ice, and transported to Kansas State University. Samples were stored frozen (-20 °C) until microhistological analysis was performed.

Microhistology. Sample preparation was conducted as described by Eckerle et al. (2009). Wet fecal samples were soaked overnight in 50% ethanol (v/v). After soaking, ethanol was decanted and samples were homogenized and washed with deionized H_2O through a No. 200 US-standard sieve. Samples were then re-homogenized, strained, and dried in a forced air-oven (96 h; 50 °C). Dried samples were ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen and stored in plastic bags for slide preparation (Bennett et al., 1999).

Slide preparation methods were described by Eckerle et al. (2009). Subsamples (0.5 g) of dried, ground, and washed plant fragments recovered from fecal material were soaked in deionized H_2O for 1 h. Approximately 20 mL of NaOH (0.05 M) was then added to each sample. Samples were incubated for 20 min at room temperature to destroy plant pigments. Samples were subsequently rinsed with deionized H_2O over a No. 200 US-standard sieve and then homogenized in a blender with 20 mL of deionized H_2O for 1 min. Samples were rinsed a second time with deionized H_2O over a No. 200 US-standard sieve.

Homogenized samples were placed on slides using an eyedropper, 2 drops of Hertwig's solution were applied, and slides were placed over a propane flame until dry. Two drops of Hoyer's solution were added to each slide, a cover slip was mounted, and slides were placed over a propane flame to set the cover slip. The cover slip was then rimmed with Hoyer's solution to ensure a solid mount. Slides were dried in a forced-air oven (96 h; 50 °C) before viewing.

Individual forage-plant species previously identified as having significance in diets of beef cattle grazing in the Kansas Flint Hills (Eckerle et al., 2009; Aubel et al. 2011) were sampled before the initiation of the trial for the purpose of developing standard slides. These species included big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), sideoats grama (*Bouteloua curtipendula*), blue grama (*Bouteloua gracilis*), switchgrass (*Panicum virgatum*), indiangrass (*Sorghastrum nutans*), leadplant (*Amorpha canescensi*), heath aster (*Symphyotrichum ericoides*), dotted gayfeather (*Liatris punctata*), purple prairie clover (*Dalea purpurea*), and sericea lespedeza (*Lespedeza cuneata*). Each sample was verified as a pure forage sample before being dried in a forced air-over (96 h; 50 °C), weighed, and ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen.

Sample slides and standards were viewed on a compound microscope at 10× magnification. The microscope was equipped with a digital camera; 20 randomly-selected slide fields from each sample and each standard were photographed (Eckerle et al., 2009).

Photographs of standard-slide fields were used to train observers to recognize histological characteristics of key plant species. Observers were required to achieve an acceptable repeatability (≤ 4% CV for each species) of plant-fragment identification relative to trainers (Holecheck and Gross, 1982).

Photographs of sample slide fields were used to measure the frequency with which plant fragments appeared in beef-cow feces (Holechek and Vavra, 1981). Individual plant species were identified according to their histological characteristics using standard slides for comparison. Due to histological similarities, big bluestem and little bluestem were grouped together for the purposes of analysis.

Plant fragment prevalence in slide fields was assumed to be equivalent to prevalence in fecal samples and equivalent to % botanical composition of the diets grazed by beef cows (Sparks and Malechek, 1968). Plant fragments that were not among the 11 range-plant species for which standards were prepared were classified as either unknown grass or unknown forb.

Extraction of Condensed Tannins. Whole-plant SL samples were collected randomly from each pasture (n = 40

plants/pasture) on 6/1, 7/1, 8/1, 9/1, and 10/1. Stems were clipped 1 cm above the soil surface and sun-dried at the collection site for subsequent analysis of condensed tannins (CT) and CT protein-binding capacity. At the laboratory, SL stems were dried in a forced air-oven (96 h; 50 °C), weighed, ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) to pass a 1-mm screen, and composited within collection date.

Extraction of CT was conducted as described by Makkar (2003). Each ground SL sample was thoroughly mixed and a 200 mg subsample was collected. Ten mL of 50% methanol (v/v) was added to each sample in a 50-mL beaker and the mixture was stirred. Samples were agitated in an ultrasonicator (Blackstone Ultrasonics, Sheffield, PA) for 2 × 10-min periods. Samples were allowed to stand for a period of 5 min between agitations. The resulting solution was transferred to 15-mL polyethylene tubes and centrifuged at 3,000 × g (4 °C) for 15 min. The supernatant was decanted into a 50-mL beaker and chilled; the pellet was washed 2× with 5 mL of 50% methanol (v/v). The centrifugation step was repeated after each wash and supernatant was decanted into the aforementioned 50-mL beaker. All supernatant from each collection date was stored frozen (-20 °C) until further CT analysis was completed.

Measurement of Condensed Tannins. Methods used for measuring CT in harvested SL were conducted as described by Makkar (2003). A 0.5-mL aliquot of supernatant from each sample was placed into individual 5-mL conical tubes; 3.0-mL of butanol-HCl and 100 µL of ferric-chloride reagent were added and the tubes were vortexed. Samples were incubated for 60 min in a 100-°C water bath. Samples were allowed to cool and then placed into a 96-well microplate. Sample absorbance was measured at 550 nm using a UV spectrophotometer equipped with Gen5 software (Biotech Inc., Winooski, VT). Absorbance was adjusted to CT concentration using leucocyandin as a standard.

Measurement of Protein-Precipitable Phenolics. Estimation of CT protein-binding capacity in harvested SL was conducted as described by Makkar (2003). Standards (1 mL) containing 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mL tannic acid in 50% methanol (v/v) were prepared from a standard solution (0.5 mg tannic acid/mL in 1% SDS); 3-mL SDS-TEA and 1-mL ferric-chloride reagent were added to each standard (total volume = 5 mL). Absorbance was measured at 510 nm using a UV spectrophotometer equipped with Gen5 software (Biotech Inc., Winooski, VT).

Tannin-protein complexes were constructed by mixing aliquots of extracted CT (0.1, 0.2, 0.3, 0.4, and 0.5 mL) with 2-mL of bovine serum albumin solution (BSA; 100 mg BSA in 100 mL acetate buffer); enough 50% methanol (v/v) was added to bring the total volume to 3 mL and the solution was vortexed.

Samples were allowed to stand at 4 °C for 16 h and then centrifuged for 10 min at 3000 x g (4 °C). Supernatant was discarded and the pellet was dissolved in 1.5 mL of 1% SDS. A 1-mL aliquot was removed from each sample and mixed with 3-mL of SDS-TEA and 1-mL of ferric-chloride reagent. Iron in the form of ferric chloride reacted with tannin phenolics to express a pink chromatophore that was measurable spectrophotometrically (Makkar, 2003). The resulting solution was allowed to stand at room temperature for 30 min before being placed into a 96-well microplate.

Absorbance was measured at 510 nm. Absorbance was then converted to the tannic-acid equivalent, using the standard curve. Values were multiplied by 1.5 (each sample was dissolved in 1.5 mL of 1% SDS solution) to calculate the amount of tannin in the tannin-protein complex (Makkar, 2003). A linear regression between tannins precipitated (as a tannic-acid equivalent) and mg of SL in the original aliquot was constructed. Protein-precipitable phenolics were represented by the slope of the line.

Total phenolics in SL were measured by mixing aliquots of extracted CT (0.1, 0.2, 0.3, 0.4, and 0.5 mL) and enough 1% SDS solution to make a 1-mL final volume. This solution was added to 3 mL SDS-TEA and 1 mL ferric-chloride reagent in a 5-mL conical vial and vortexed. Absorbance was read immediately thereafter at 510 nm; absorbance was converted to a tannic-acid equivalent using the standard curve. A linear regression between tannic-acid equivalent and mg of SL in the original aliquot was constructed. Total phenolics were represented by the slope of the line.

The percentage of total phenolics which precipitated protein (i.e. protein-binding capacity) was calculated as *protein-precipitable phenolics* divided by *total phenolics*. Protein binding capacity was represented by: (slope X / slope Y) * 100 (Makkar, 2003).

Statistical Analysis. Microhistology data were analyzed separately for each plant species or plant group (e.g., grasses). Data were analyzed as a generalized mixed model with a 1-way treatment structure in a completely-randomized design with subsampling and a split-plot in time (PROC GLIMMIX; SAS Inst. Inc., Cary, NC), using the binomial distribution and logit-link function. Pasture was the experimental unit and animal was the subsample unit. Class factors included animal, pasture, treatment, and collection date. The model statement included terms for the fixed effects of treatment, collection date, and treatment × collection date, which were tested using type-3 F tests. The random statement had terms for pasture within treatment, animal within pasture and treatment, and collection date × pasture within treatment.

Concentration and protein binding capacity of CT in SL were analyzed as a mixed model with a 1-way treatment structure in a completely-randomized design with a split-plot in time (PROC MIXED; SAS Inst. Inc., Cary, NC). Pasture was the experimental unit. Class factors included pasture, treatment, and collection date. The model statement included terms for the fixed effects of treatment, collection date, and treatment × collection date, which were tested using type-3 F tests. The random statement had a term for pasture within treatment.

Treatment main effects and treatment × collection period effects were not detected ($P > 0.05$) for CT concentrations in SL, protein binding capacity of CT in SL, or for 10 of the 11 plant species examined in the microhistological portion of the study; therefore, main effects of collection date were reported. Conversely, prevalence of SL in beef cow diets was influenced ($P < 0.01$) by CSL supplementation and by collection date;

therefore, treatment × collection date means were presented. Interaction effects were tested using collection date contrasts (PROC GLIMMIX, SAS Inst. Inst., Cary, NC) according to the following equation: $(\mu_{supplemented,\ June} - \mu_{unsupplemented,\ June}) - (\mu_{supplemented,\ date\ x} - \mu_{unsupplemented,\ date\ x}) = 0$.

Least Squares collection-date main effect means were separated using the method of Least Significant Difference when protected by a significant F-test ($P < 0.05$). Means were considered different when $P \leq 0.05$. Tendencies were discussed when $0.05 < P \leq 0.10$.

Results and Discussion

CT Analysis. Concentration of CT in SL increased ($P < 0.01$) as the grazing season advanced and reached a peak during the 8/1 collection (Table 1). Thereafter, CT concentration declined. Protein-binding capacity of CT in SL generally mirrored CT concentration; however, peak protein-binding capacity occurred one month later (9/1) than peak CT concentration. Our values for CT in dried SL were greater than those reported by Eckerle et al. (2010). Weather patterns during our study were characterized by below-normal precipitation and above-normal temperatures (Preedy et al., 2013). Donnelly (1959) indicated that CT in SL increased with both above-normal temperature and below-normal rainfall.

Table 1. Effect of harvest date on condensed tannins in sericea lespedeza.

Item	Condensed tannins, g/kg	Protein binding capacity, %
June 1	103.9[a]	46.2[a]
July 1	151.1[b]	45.5[a]
August 1	191.1[d]	49.2[c]
September 1	169.4[c]	52.3[d]
October 1	145.4[b]	47.9[b]
SEM	1.05	0.15

[a] Within a column, means without a common superscript differ ($P < 0.01$).
[b] Percentage of total phenolic compounds which precipitated proteins.

Microhistology. Prevalence of SL in beef cow diets was influenced ($P < 0.01$) by CSL supplementation and by collection date (Figure 1). Although SL selection by CSL-supplemented beef cows was numerically greater than that by unsupplemented beef cows at each of the 5 collection dates, prevalence of SL in beef cow diets was sufficiently variable that no difference ($P \geq 0.55$) between treatments was detected when concentration and protein-binding capacity of CT were relatively low (6/1, 7/1, and 10/1; Table 1). Conversely, CSL-supplemented cows selected 30% more ($P < 0.01$) SL during the 8/1 collection than unsupplemented cows and 49% more ($P < 0.01$) SL during the 9/1 collection than unsupplemented cows. These times corresponded to greatest CT concentration and CT protein-binding capacity in SL.

We reported previously that supplemental CSL fed at a rate of 0.6 kg DM/cow daily alleviated the negative effects of CT on DMI by beef cows fed prairie hay contaminated with SL; total-tract digestion of CP and digestible DMI were normalized when CSL was fed at 1.2 to 1.8 kg DM/cow daily (Eckerle et al., 2011b). In addition, beef cows supplemented with CSL at 0.6 kg DM/d did not discriminate between SL-contaminated and SL-free prairie hay in a preference trial, whereas unsupplemented beef cows displayed strong preference for SL-free prairie hay over SL-contaminated prairie hay (Eckerle et al., 2011c).

The relative abundance of SL in the diets of beef cows in our study ranged from a low of 3.5% (unsupplemented cows on 9/1) to a high of 7.5% (CSL-supplemented cows on 10/1). We interpreted this to indicate that, under the conditions of our study, SL was an important forb component of the diet in both supplemented and unsupplemented beef cows. The significance of increased voluntary selection of SL by CSL-supplemented beef cows during August and September was that these months corresponded to flowering and seed production by SL in the Kansas Flint Hills. Increased grazing pressure achieved with goats during this interval of time resulted in drastically-reduced seed production by SL (Mayo, 2000; Hart, 2001).

Supplemental CSL had no influence ($P \geq 0.38$) on voluntary selection of other individual plant species that were examined in our study; however, there were important temporal differences in selection of various ecologically-important grasses and forbs. Leadplant was not detected in fecal samples and was, therefore, not discussed.

Displayed preferences for individual forage plants are influenced by herbivore perceptions of palatability, plant growth form, plant nutrient composition, and herbivore experience; moreover, preferences may change dramatically over time in native-range production systems due to temporal fluctuations in availability of key species and maturity-driven changes in plant palatability, growth form, and nutrient content (Vallentine, 1990; Holecheck et al., 2001). In general, there were 3 distinct temporal responses in voluntary selection by beef cows of the plant species we examined in our study: a decrease over time, an increase over time, or an increase from the beginning to the midpoint of the study and decline thereafter.

Voluntary selection of all grass species (Figure 2), big bluestem + little bluestem, indiangrass (Figure 3), and unidentified grasses (Figure 4) decreased ($P < 0.01$) from 6/1 to 10/1. We speculated that selection of these species was inversely related to nutrient content, as native tallgrasses tend to have excellent nutrient profiles while vegetative; however, quality rapidly declines as plant maturity advances (Rao, 1972, Umoh, 1977). Aubel et al. (2011) reported similar trends among beef cows grazing annually-burned native range in the Kansas Flint Hills.

During the same interval, voluntary selection of all forbs (Figure 2), switchgrass, blue grama, heath aster (Figure 5), and unidentified forbs (Figure 4) increased ($P < 0.05$). In general, the magnitude of change in forb selection from the beginning to the end of the grazing season was greater than that reported by McGinty et al. (1983) and Aubel et al. (2011). The abnormally warm, dry conditions under which our study was conducted may have influenced both the availability and quality of the forb plants we monitored.

Voluntary selection of sideoats grama, purple prairie clover, and dotted gayfeather was relatively low at the outset of the study (Figure 6). Selection of each increased as the grazing season advanced, reached a peak in August or September, and then declined thereafter. Aubel et al.

(2011) reported a similar temporal pattern in selection of these 3 species but much greater prevalence in the diets of beef cows grazing native tallgrass range during a non-drought year. The drought conditions under which our study was conducted likely influenced the availability and palatability of most plant species; moreover, there were notable differences in plant-species composition between their research site and ours (Towne and Owensby, 1984).

Implications

Supplemental CSL increased beef cow tolerance for and acceptance of high-CT SL in a commercial-scale, native-range production system. We concluded that supplemental CSL allowed for a desirable change in selection preference by beef cows that stemmed from a critical modification of the post-ingestive consequences associated with CT consumption. The significance of increased voluntary selection of SL by CSL-supplemented beef cows during August and September was that these months corresponded to flowering and seed production by SL in the Kansas Flint Hills. Increased grazing pressure during this interval of time may result in drastically-reduced seed production by SL.

Supplemental CSL did not influence voluntary selection by beef cows of any other forage-plant species monitored in our study; however, there were noteworthy temporal shifts in selection. We speculated that these shifts in voluntary selection were driven by changes in plant availability, changes in nutrient composition, or both.

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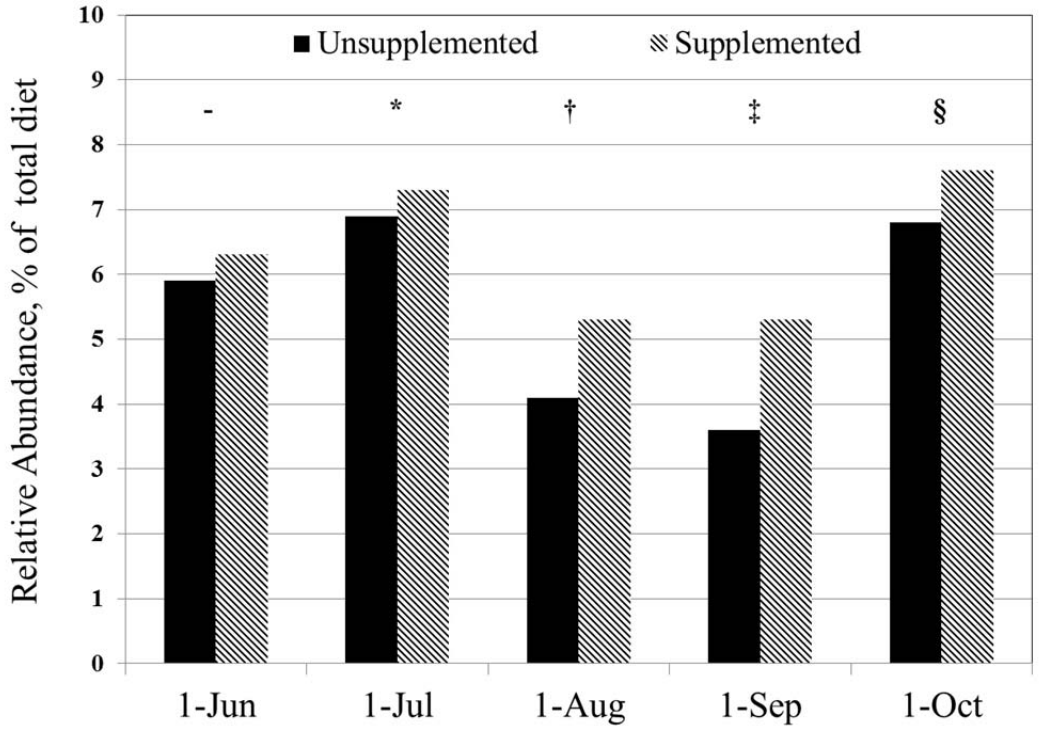
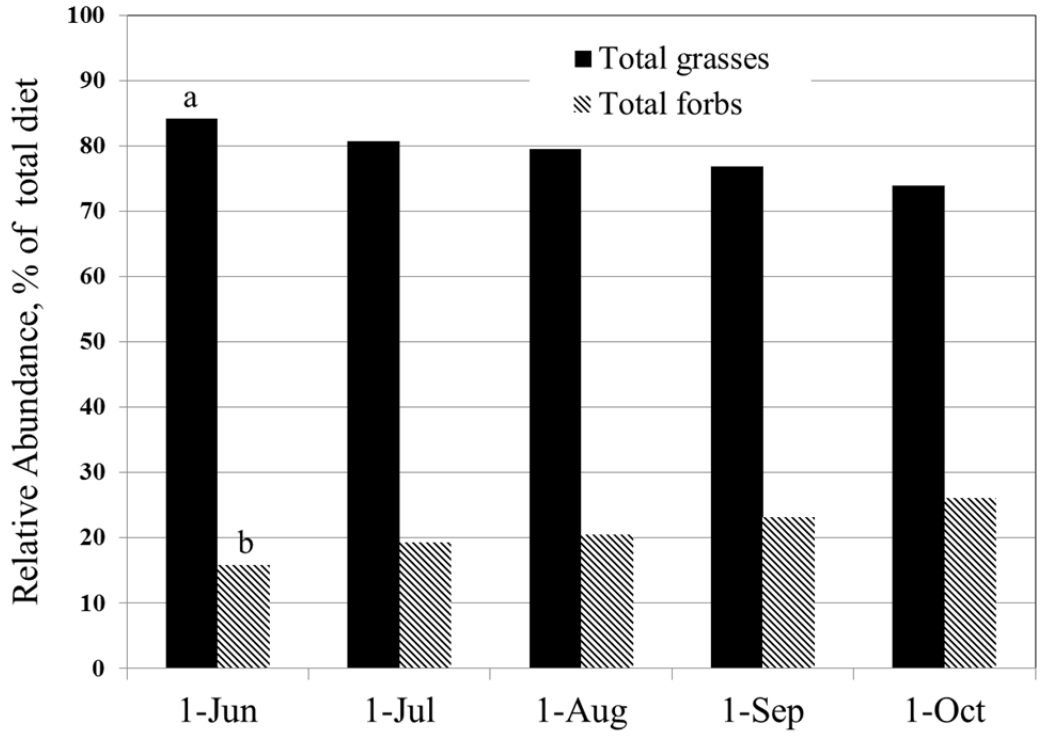
Figure 1. Effects of corn steep liquor supplementation on the relative abundance of sericea lespedeza in diets of beef cows grazing native range in the Kansas Flint Hills.



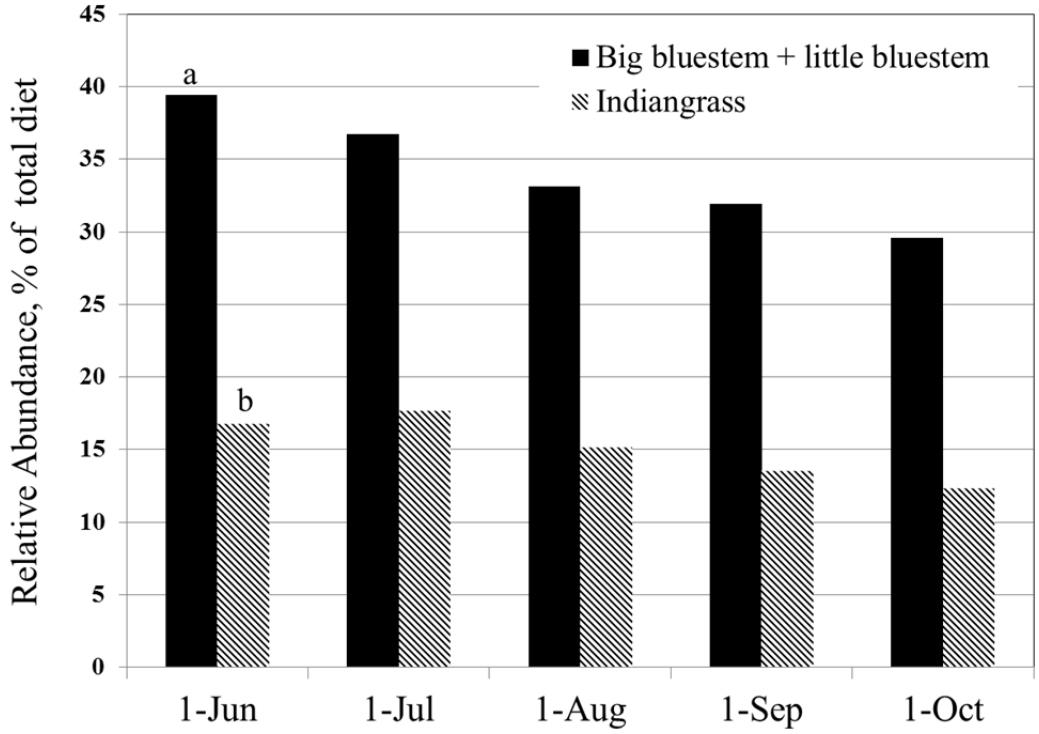
* Contrast: ($\mu_{supplemented\text{-}June} - \mu_{unsupplemented\text{-}June}$) - ($\mu_{supplemented\text{-}July} - \mu_{unsupplemented\text{-}July}$) = 0 – ($P = 0.93$).
† Contrast: ($\mu_{supplemented\text{-}June} - \mu_{unsupplemented\text{-}June}$) - ($\mu_{supplemented\text{-}August} - \mu_{unsupplemented\text{-}August}$) = 0 – ($P < 0.01$).
‡ Contrast: ($\mu_{supplemented\text{-}June} - \mu_{unsupplemented\text{-}June}$) - ($\mu_{supplemented\text{-}September} - \mu_{unsupplemented\text{-}September}$) = 0 – ($P < 0.01$).
§ Contrast: ($\mu_{supplemented\text{-}June} - \mu_{unsupplemented\text{-}June}$) - ($\mu_{supplemented\text{-}October} - \mu_{unsupplemented\text{-}October}$) = 0 – ($P = 0.35$).

Figure 2. Relative abundance of grasses and forbs in diets of beef cows grazing native range in the Kansas Flint Hills.



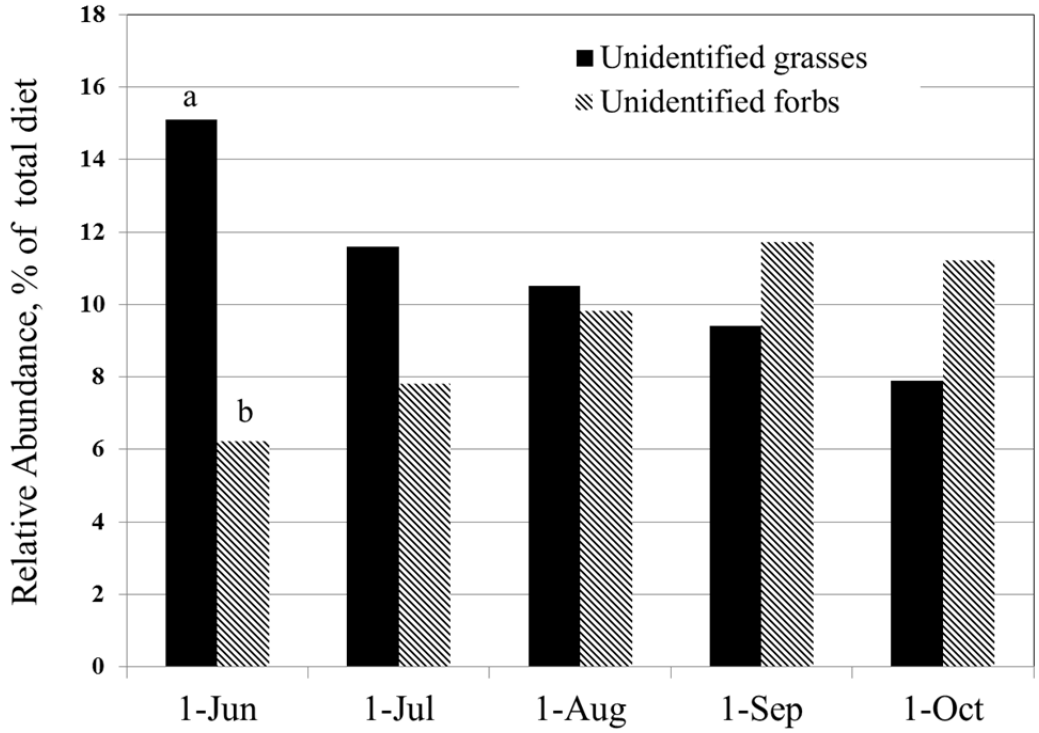
[a] Effect of time on selection of all graminoid species (Quartic – $P < 0.01$).
[b] Effect of time on selection of all forb species (Quartic – $P < 0.01$).

Figure 3. Relative abundance of bluestem *spp.* and indiangrass in diets of beef cows grazing native range in the Kansas Flint Hills.



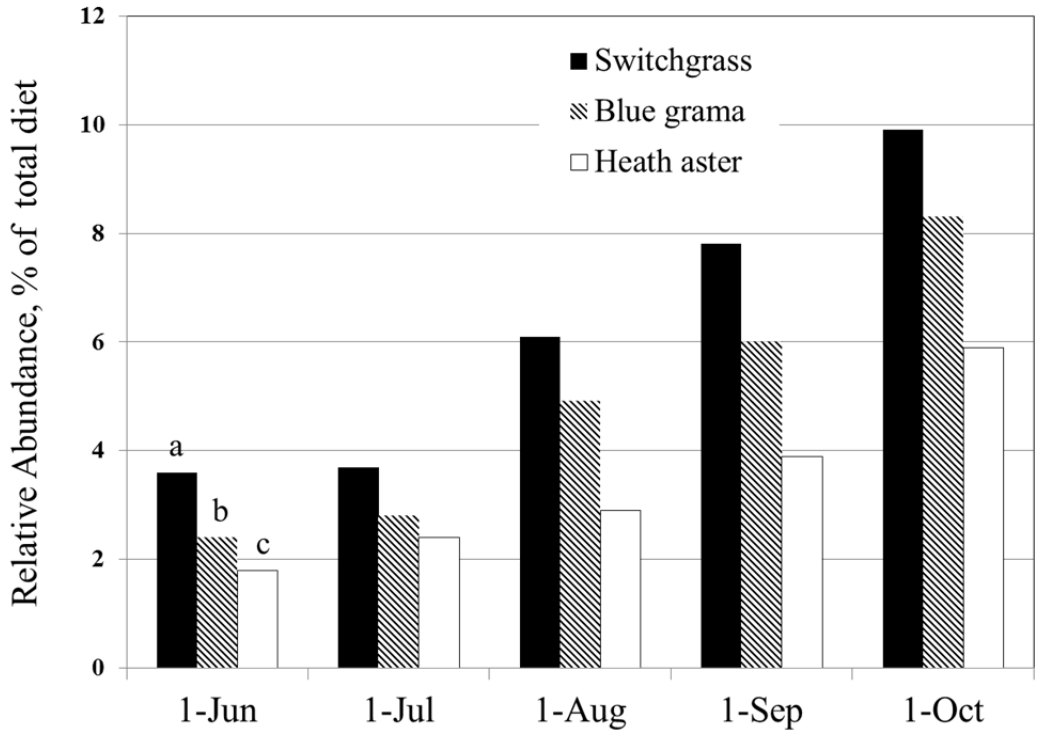
[a] Effect of time on selection of big bluestem and little bluestem (Quartic – $P < 0.01$).
[b] Effect of time on selection of indiangrass (Quartic – $P < 0.01$).

Figure 4. Relative abundance of unidentified grasses and unidentified forbs in diets of beef cows grazing native range in the Kansas Flint Hills.



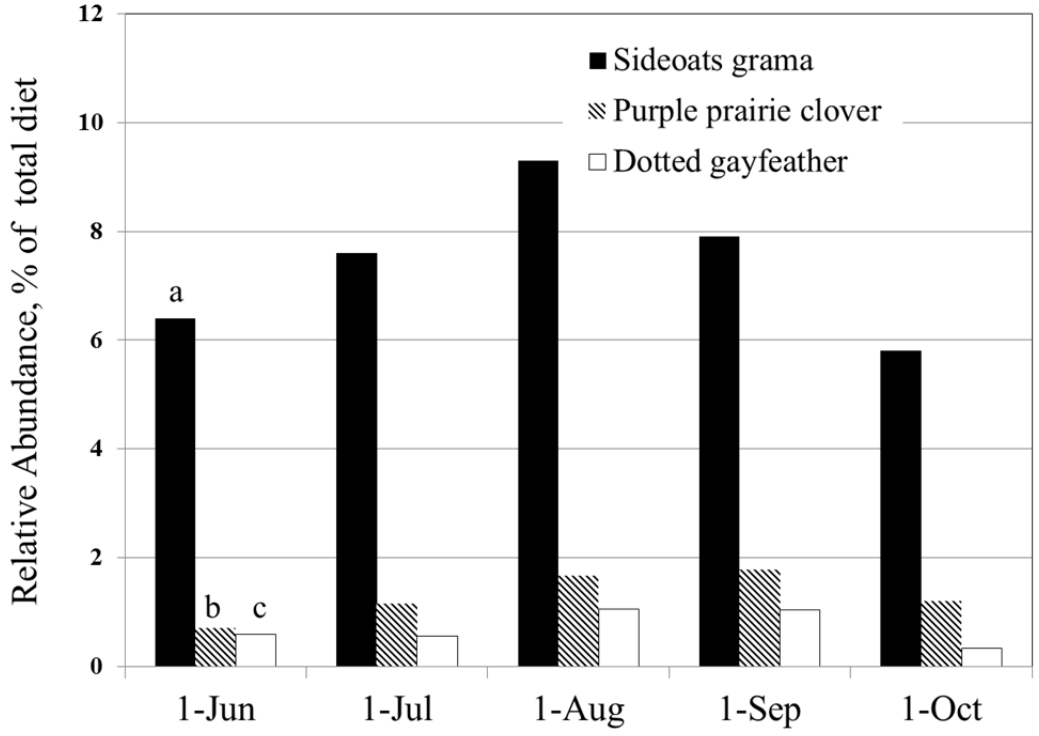
[a] Effect of time on selection of unidentified graminoid species (Cubic – $P < 0.01$).
[b] Effect of time on selection of unidentified forb species (Cubic – $P < 0.01$).

Figure 5. Relative abundance of switchgrass, blue grama, and heath aster in diets of beef cows grazing native range in the Kansas Flint Hills.



[a] Effect of time on selection of switchgrass (Quartic – $P < 0.05$).
[b] Effect of time on selection of blue grama (Quartic – $P < 0.01$).
[c] Effect of time on selection of heather aster (Cubic – $P < 0.05$).

Figure 6. Relative abundance of sideoats grama, purple prairie clover, and dotted gayfeather in diets of beef cows grazing native range in the Kansas Flint Hills.



[a] Effect of time on selection of sideoats grama (Quartic – $P < 0.01$).
[b] Effect of time on selection of purple prairie clover (Cubic – $P < 0.05$).
[c] Effect of time on selection of dotted gayfeather (Cubic – $P < 0.05$).

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CINNAGAR SUPPLEMENTATION OF CATTLE GRAZING WHEAT OR NATIVE PASTURE IN NORTHWEST OKLAHOMA [1]

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ABSTRACT: Two experiments were conducted to evaluate CinnaGar (Provimi; Trappes, France) and monensin (Ruminsen; Elanco Animal Health, Greenfield, IN) as supplements for grazing stocker cattle in northwest Oklahoma. In Exp. 1, twelve 2.2-ha pastures of winter wheat were grazed (1.2 steers/ha) with stocker steers (initial BW = 250 kg) starting March 3 for 70 d. Pastures were fertilized in early September with 56 kg of N/ha from urea. Free-choice minerals (Beef Minerals 3V6S; Vigortone Ag Products, Brooksville, OH) were provided to 6 pastures in ground-style mineral feeders, while the other pastures received a similar mineral (Beef Minerals 3V6SG, Vigortone Ag Products) except it contained 1,587 g/tonne of CinnaGar. In Exp. 2, 16 native pastures of sand sagebrush rangeland (between 4.1 and 8.2 ha each) were stocked (36 animal-unit-d/ha) with steers (initial BW = 254 kg) starting April 24 for 84 d. A base free-choice mineral (Beef Mineral 3V6S) was used and monensin and CinnaGar were added in a 2 x 2 factorial arrangement of treatments; 1) 0 and 0; 2) 1,761 and 0; 3) 0 and 2,351; and 4) 1,761 and 2,351 g/tonne of monensin and CinnaGar, respectively. In Exp. 1, mineral intake was 150 and 107 g/d for no CinnaGar and CinnaGar (P = 0.24), respectively. Also, ADG (overall avg = 1.4 kg) did differ (P = 0.66) between no CinnGar and CinnaGar; hence, ending BW (overall avg = 339 kg) or BW gain/ha (overall avg = 44 kg) did not differ (P > 0.52). In Exp. 2, mineral intake differed (P < 0.01) by treatment and was 79, 31, 68, and 43 g/d, respectively; the addition of monensin decreased mineral intake (P < 0.01), while CinnaGar did not affect mineral intake (P = 0.80). Further, ADG (overall avg = 0.65 kg) did not differ (P = 0.47) among treatments; hence, ending BW (overall avg = 305 kg) or BW gain/ha (overall avg = 41 kg) did not differ (P > 0.48). At these levels of CinnaGar intake and with these types of pasture, these supplements did not significantly affect animal performance.

Key Words: Steers, Monensin, Wheat pasture, Rangeland

Introduction

Mineral supplementation for cattle grazing wheat (*Triticum aestivum* L) pasture can significantly affect the net return to the producer (Horn et al., 2002; Gunter and Combs, 2010). The mineral composition of wheat forage indicates that it provides inadequate Ca for cattle (Stewart et al., 1981; Grunes et al., 1983). With cattle grazing sand sagebrush rangeland (Berg, 1994) in the southern Great Plains, research has proven that forages are adequate in P and Ca (Savage and Heller, 1947), unlike results from tallgrass prairie (Glendening et al., 1952; Umoh et al., 1982). However, we are unaware of research examining the adequacy of these pasture types with respect to other essential minerals.

Free-choice minerals are commonly used as supplements for grazing cattle. Often, supplements also contain other components such as garlic extracts and essential oils, which have been shown to improve ruminal fermentation by increasing the propionate to acetate ratio (Busquet et al., 2005; Wanapat et al., 2008), and cinnamaldehyde, which has been shown to decrease the ruminal degradability of feed proteins like those found in wheat forage (Busquet et al., 2005; Yang et al., 2010).

We conducted 2 experiments to determine the efficacy of mineral supplementation in the presence of monensin and a mixture containing both garlic extract and cinnamaldehyde (CinnaGar, Provimi; Trappes, France) for cattle grazing wheat pasture or sand sagebrush rangeland in Northwest Oklahoma.

Materials and Methods

The experimental design and all animal procedures were approved by the USDA-ARS Southern Plains Range Research Station Institutional Animal Care and Use Committee.

In Exp. 1, twelve 2.5-ha pastures on the Southern Plains Experimental Range near Ft. Supply, OK were planted to wheat (67 kg/ha of seed) the first 2 wk of September 2009. Four of the pastures were planted by offset disking to prepare a seedbed in early June, followed by harrowing as needed to control weeds. Pastures were fertilized before the final harrowing during the last week of August according to soil test recommendations for N, P, and K from the Oklahoma State University Soil and Water Testing Laboratory (Stillwater, OK) with 50 kg/ha of N from urea. The remaining 8 pastures were planted using a no-till drill (Model 750; John Deere, Moline, IL); weeds had previously been controlled by glyphosate application. No-till pastures were fertilized by top dressing with 50 kg/ha of N from urea 2 wk after seedling emergence.

On March 3, pastures were stocked with 6 steers (initial BW = 250 ± 3.9 kg) on each of the pastures (1.0 steers/ha) and were removed on May 12 (70 d). Half the pastures were selected randomly and were offered a free-choice mineral supplement (3V6S Beef Minerals;

[1]This project was conducted in part with a gift from Provimi North America, Inc. (Brooksville, OH). Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

Vigortone Ag Products, Brookville, OH) in ground-type mineral feeders (Sioux Steel Company, Sioux City, SD). The mineral mixture contained (as-fed) 19 to 22% Ca, 3.0% P, 1.0% Mg, 18.2 to 21.8% NaCl, 662,000 IU of vitamin A (as retinyl palmitate)/kg, and trace minerals (1,000 ppm of Cu from copper chloride; 3,750 ppm of Zn from zinc oxide; 26.4 ppm Se from sodium selenite). The other pastures received a similar mineral mixture (3V6SCG Beef Mineral containing CinnaGar at 1,587 g/tonne). Cattle received no additional salt or supplement of any kind. Mineral feeders were weighed weekly to determine mineral intake, with any foreign matter being discarded before weighing. On d 0, 35, and 70, calves were weighed following a 16-h withdrawal of feed and water. Herbage mass samples were collected on weigh days at 10 paced transects in each pasture by clipping forage to the ground on 2 sides of a 61-cm rod placed between drill rows. Herbage was placed in paper sacks, dried in a forced-air oven at 56°C to determine DM, and ground in a Wiley mill to pass a 1-mm screen before analysis for total N (Vario MAX CN; Elementar Ammericas, Inc., Mt. Laurel, NJ). The forage CP was calculated by multiplying N concentration by 6.25, ash (AOAC, 1990), and in vitro OM disappearance (**IVOMD**; White et al., 1981). Minerals were determined after nitric-perchloric acid hydrolysis by inductively coupled argon plasma emission spectrophotometry (Ca P, Mg, K, S, Na, Fe, Zn, Cu, Mn, Mo) or electrothermal atomic absorption spectrophotometry using Zeeman-effect background correction (Se).

In Exp. 2, 132 steer and heifer calves (249 ± 4.7 kg) were stratified by gender and BW then randomly assigned to the 16 pastures. Of the initial group of calves, 105 (BW = 254 ± 2.9 kg) were selected to be used based on initial BW. Pastures were also stratified by the number of sand sagebrush plants/ha (High, 242; Low, 12); the treatments were randomly assigned to each pasture so treatments were equally distributed across sagebrush densities. The free-choice mineral types fed were as follows: 1) Beef Mineral (3V6S, Vigortone Ag Products); 2) Beef Mineral containing 1,791 g/tonne of monensin (Rumensin; Elanco Animal Health, Greenfield, IN), 3) Beef Mineral containing 2,351 g/tonne of CinnaGar, or 4) Beef Mineral containing 1,791 g/tonne of Rumensin and 2,351 g/tonne of CinnaGar. The concentration of CinnaGar was selected after consultation with Vigortone Ag Products technical staff (Randy Dew; Muscatine, IA). Mineral mixtures were offered in ground-style mineral feeders and managed as described in Exp. 1.

Cattle grazed the pastures 84 d from April 24 to July 17, 2012. The cattle were supplemented with 1 kg of a cottonseed meal-based supplement (42% CP on a DM basis)/steer offered 3 d weekly. On weigh days, cattle were gathered and shrunk over night (no feed or water for 16 h) before weighing the following morning (d 0, 56, and 84) starting at 0730. Also on cattle weigh days, standing forage mass in each pasture was determined by clipping the forage to the ground inside twenty 0.10-m^2 quadrants along paced transects. Forage samples were dried in a forced-air oven (56°C) to determine DM, weighed, amalgamated, ground to pass a 2-mm screen, and were analyzed for IVOMD, CP, ADF, NDF, Ca, P, Mg, K, S, Na, Fe, Zn, Cu, and Mn as described in Exp. 1.

Table 1. Performance of stocker cattle grazing wheat pasture supplemented with free-choice minerals near Ft. Supply, OK (Exp. 1)

	Mineral supplement[a]			
Item/period	Control	CinnaGar[b]	SE	*P*-value
BW, kg				
d 0	250	250	4.5	0.95
d 35[c]	286	287	2.9	0.72
d 70[c]	338	340	6.1	0.82
ADG, kg				
First 35 d	1.0	1.1	0.08	0.72
Second 35 d	1.6	1.6	0.09	0.96
Overall	1.4	1.3	0.06	0.66
BW gain/ha, kg	113	106	11.3	0.67
Herbage mass, kg of DM/animal				
D 0	1,010	1,155	156.3	0.53
D 42	1,042	620	150.2	0.09
D 84	1,102	494	223.5	0.17
Forage CP, % of OM				
D 0	24.4	23.8	0.88	0.69
D 42	23.8	24.4	0.69	0.61
D 84	13.1	14.4	1.50	0.62
Forage IVOMD[d], %				
D 0	56.9	58.2	6.56	0.88
D 42	72.9	72.2	1.54	0.74
D 84	70.3	71.0	1.03	0.63
Total mineral intake, g/d as-fed				
First 35 d	96	77	14.5	0.90
Second 35 d	204	159	43.7	0.36
Overall, 70 d	159	99	25.0	0.24

[a]Free-choice mineral (Beef Minerals 3V6S, Vigortone Ag Products; Brooksville, OH) supplied in ground-style mineral feeders (Sioux Steel Co.; Sioux City, SD).

[b]1,587 g/tonne of CinnaGar (Provimi; Trappes, France) added.

[c]Least squares means were adjusted for BW on d 0 as a covariate ($P < 0.05$).

[d]In vitro OM disappearance.

Statistical Analyses. In Exp. 1, BW, ADG, BW gain/ha, mineral intake, herbage mass, and forage nutritive values were analyzed using the MIXED procedure in SAS (SAS Inst., Inc.; Cary, NC). The model included mineral treatment and tillage method as fixed effects and pasture as a random effect; tillage method was insignificant in all analysis ($P > 0.10$; tillage effects not presented). Models for BW on d 35 and 70 included initial BW as a covariate ($P < 0.05$). In Exp. 2, data were analyzed using the MIXED procedure in SAS (SAS Inst., Inc.) with monensin, CinnaGar, and their interaction as fixed effects and pasture as the random effect; sagebrush plants per hectare were included in the initial model as a covariate. Sagebrush plants per hectare were a non-significant ($P \geq 0.10$) source of variation; hence, it was removed from the model. In both experiments, least-square means were separated using contrasts.

Results and Discussion

In Exp. 1, initial BW (d 0) did not differ between treatments (Table 1). Further, after 42 and 84 d of grazing, BW still did not differ between treatments. Average daily gain during the first and second 42-d periods did not differ between treatments (Table 1). Also, ADG between treatments did not differ during the entire 84-d grazing period. Body weight gain per hectare did not differ between cattle fed control minerals versus minerals containing CinnGar. Standing herbage mass did not differ between treatments (Table 1), and CP, IVOMD, NDF ($P \geq 0.19$; 42.5 ± 0.64, 51.6 ± 0.72, and 60.2 ± 0.58% of OM for d 0, 42, and 84, respectively), and ADF ($P > 0.19$; 18.3 ± 0.30, 26.1 ± 0.57, and 31.5 ± 0.44% of OM for d 0, 42, and 84, respectively) concentrations in forage samples did not differ between treatments on any sampling day. Minerals in herbage samples did not differ ($P > 0.11$) between treatments (data not shown), but mineral concentrations did change over time. On March 3 (d 0), mineral concentrations in DM were Ca = 0.38 ± 0.047%, P = 0.15 ± 0.012%, Mg = 0.15 ± 0.008%, K = 1.8 ± 0.05%, S = 0.25 ± 0.011%, Na = 0.016 ± 0.0105%, Fe = 997 ± 111.3 ppm, Zn = 20 ± 1.2 ppm, Cu = 5 ± 0.2 ppm, Mn = 123 ± 16.4 ppm, Mo = 0.98 ± 0.417 ppm, and Se = 0.19 ± 0.073 ppm. On d 42, the mineral concentrations were Ca = 0.46 ± 0.005, P = 0.17 ± 0.014%, Mg = 0.16 ± 0.011%, K = 1.7 ± 0.10%, S = 0.26 ± 0.008%, Na = 0.012 ± 0.0072%, Fe = 980 ± 157.7 ppm, Zn = 20 ± 1.6 ppm, Cu = 5 ± 0.2 ppm, Mn = 88 ± 7.5 ppm, Mo = 1.58 ± 0.556 ppm, and Se = 0.28 ± 0.011 ppm. At the end of the grazing period (d 84), the mineral concentrations were Ca = 0.43 ± 0.075, P = 0.29 ± 0.015%, Mg = 0.15 ± 0.015%, K = 2.3 ± 0.11%, S = 0.25 ± 0.011%, Na = 0.008 ± 0.0043%, Fe = 788 ± 278.0 ppm, Zn = 27 ± 2.8 ppm, Cu = 6 ± 0.2 ppm, Mn = 79 ± 7.2 ppm, Mo = 1.24 ± 0.437 ppm, and Se = 0.23 ± 0.083 ppm. Total mineral intake did not differ between treatments and averaged 129 g/d, which was well within the manufacturer's recommendation of near 113 g/d.

In Exp. 2, initial BW (d 0) did not differ among mineral additives (Table 2). After 56 and 84 d of grazing, BW still did not differ among mineral additives. Hence, ADG and BW gain per hectare did not differ among monensin or CinnaGar addition. Standing herbage mass did not differ between cattle fed minerals with monensin or CinnaGar on any day. Further, forage CP, IVOMD, NDF ($P \geq 0.34$; 61.5 ± 1.15, 65.7 ± 2.48, and 60.8 ± 2.98% of OM for d 0, 56, and 70, respectively), or ADF ($P > 0.14$; 42.7 ± 1.64, 45.5 ± 0.84, and 47.2 ± 1.22% of OM for d 0, 56, and 70, respectively) concentrations did not differ among treatments. The mineral concentrations in the forage did not differ ($P \geq 0.12$) by treatment or by day of

Table 2. Performance of stocker cattle grazing sand sagebrush rangeland supplemented with free-choice minerals near Ft. Supply, OK (Exp. 2)

	Mineral supplement[a]					*P*-value[b]		
Item/day or period	3V6S	3V6SC	3V6SR	3V6SCR	SE	C	M	M x C
BW, kg								
D 0	253	252	257	249	5.7	0.31	0.98	0.39
D 56[c]	288	290	296	289	3.6	0.64	0.29	0.20
D 84[c]	303	303	308	307	3.3	0.80	0.13	0.85
ADG, kg								
First 56 d	0.6	0.7	0.8	0.6	0.06	0.58	0.28	0.17
Second 28 d	0.5	0.5	0.5	1.4	0.30	0.79	0.72	0.17
Overall, 84 d	0.6	0.6	0.7	0.7	0.04	0.71	0.12	0.78
BW gain/ha, kg	25	25	28	25	1.8	0.43	0.27	0.48
Herbage mass, kg of DM/ha								
D 0	1,163	1,351	1,226	1,374	121.6	0.35	0.96	0.81
D 56	2,012	1,603	1,891	1,762	371.4	0.41	0.95	0.67
D 84	1,597	1,854	1,794	1,702	187.3	0.67	0.90	0.37
Forage CP, % of OM								
D 0	12.0	10.0	12.6	11.0	0.77	0.04	0.32	0.81
D 56	6.2	6.0	6.0	2.9	0.32	0.74	0.62	0.84
D 84	5.8	6.4	6.3	6.5	0.57	0.46	0.64	0.74
Forage IVOMD[d], %								
D 0	61.4	58.2	62.2	57.9	2.06	0.09	0.91	0.77
D 56	51.9	50.6	49.8	49.3	0.94	0.34	0.09	0.66
D 84	46.1	48.3	48.2	47.7	1.46	0.55	0.62	0.38
Mineral intake, g/d as-fed								
First 56 d	98	104	26	45	8.5	0.78	< 0.01	0.02
Second 28 d	30	52	39	35	6.8	0.93	0.04	0.51
Overall, 84 d	82	68	31	41	7.1	0.80	< 0.01	0.06

[a]Free-choice mineral (Beef Minerals, Vigortone Ag Products, Brooksville, OH) supplied in ground style mineral feeders (Sioux Steel Company; Sioux City, SD).

[b]C = CinnaGar (Provimi; Trappes, France), M = monensin (Ruminsen, Elanco Animal Health; Greenfield, IN), and M x C = their interaction.

[c]Least square means were adjusted for calf BW on d 0 as a covariate ($P < 0.05$).

[d]In vitro OM disappearance.

harvest. The average concentrations were Ca = 0.94 ± 0.219%, P = 0.14 ± 0.012%, Mg = 0.20 ± 0.043%, K = 1.3 ± 0.17%, S = 0.16 ± 0.025%, Na = 0.004 ± 0.0009%, Fe = 408 ± 114.5 ppm, Zn = 36 ± 5.1 ppm, Cu = 21 ± 2.4 ppm, and Mn = 65 ± 10.3 ppm. Mineral intake interacted (P = 0.02) between monensin and CinnaGar during the first 56 d, while forage nutritive values was greater. Minerals with CinnaGar alone had the greatest intake, while minerals with only monensin added had the lowest intake; the minerals containing both monensin and CinnaGar had an intake intermediate to minerals with a single additive. During the last 28 d, mineral intake did not interact by monensin and CinnaGar. However, monensin did decrease (P = 0.4) mineral intake compared to minerals without monensin. For the entire grazing period, mineral intake did interact (P = 0.06) by monensin and CinnaGar, an effect imposed by the intake rates recorded during the first 56 d.

In Exp. 1 and 2, cattle performance was not affected by the addition of CinnaGar or Rumensin in Exp. 2. Even though cinnamaldehyde and garlic extracts have been shown to improve ruminal fermentation parameters in vitro (Busquet et al., 2005; Wanapat et al., 2008), supplementation with a product containing these materials failed to affect cattle performance in this experiment. Mineral concentrations in the wheat forage followed a pattern similar to that of forage samples collected near our research station (Stewart et al., 1981; Fieser et al., 2007). The Ca and P concentration in the forage samples from the sand sagebrush rangeland were similar to reports by Black (1943) and Savage and Heller (1947). We are unaware of other reports of the contents of other minerals in these forages. Of the minerals we measured, only Na appeared to be limiting for non-stressed cattle (NRC, 1996).

In summary, these experiments showed that additions of CinnaGar or monensin to free-choice mineral mixtures did not augment cattle performance. Further, the mineral contents of wheat forage were similar to those observed in other parts of Oklahoma. The mineral contents of forage from native rangeland indicate from early spring to mid–summer those forages are adequate to meet the mineral needs of of cattle in non-stressful production environments.

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GRAZING EFFECTS ON NEBRASKA SANDHILLS MEADOW NUTRIENT COMPOSITION

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ABSTRACT: Eight Nebraska Sandhills subirrigated meadow pastures (65ha ± 19 ha) were utilized to measure the effects of grazing on forage nutrient composition. Pastures were sampled prior to cattle grazing and after the allotted forage had been grazed. Three esophageally fistulated cows were utilized for sample collection on 4 dates (early June, late June, early July, and late July). Stocking rates were 37, 37, 74, and 47 animal unit days per ha for early June, late June, early July and late July, respectively. Samples were lyophilized and analyzed for CP, NDF, and IVDMD. Non-grazed early June pastures were higher in CP (12.0% vs. 7.1% respectively; P<0.001) and IVDMD (66.7% vs. 58.1% respectively; P=0.03) and lower in NDF (66.8% vs. 75.7% respectively; P=0.07) than grazed pastures. Late June pastures exhibited similar patterns such that non-grazed pastures were higher in CP (9.8% vs. 7.2% respectively; P=0.03) and tended to be higher in IVDMD (63.5% vs. 57.0% respectively; P=0.09) than grazed pastures. Late June NDF was numerically lower for non-grazed (70.4% vs. 78.7% respectively; P=0.11) compared to grazed pastures. Early July pastures did not differ between non-grazed vs. grazed for CP (8.7% vs. 8.3% respectively; P=0.30). Neutral detergent fiber tended to be lower (61.8% vs. 67.8% respectively; P=0.08) and IVDMD tended to be higher (58.2% vs. 54.1% respectively; P=0.09) for non-grazed compared to grazed pastures. Late July non-grazed pastures had higher CP levels (10.0% vs. 8.0% respectively; P=0.05) than grazed pastures. However, there were no differences between non-grazed vs. grazed pastures for NDF and IVDMD in late July. These data suggest grazing has the most impact on forage quality early in the grazing season.

KEY WORDS: grazing, Sandhills meadows, forage quality

Introduction

The Nebraska Sandhills subirrigated meadows are an excellent resource for grazing cattle. Most are dominated by cool-season grass species which have greater growth during early spring. However, as temperatures increase by mid-summer, forage quality decreases (Lardy et al., 2004). Previous research has shown the changes in forage nutrient composition throughout the year, but it is unclear to what degree stocking rate affects the nutrient composition of Sandhills subirrigated meadows. According to Heitschmidt et al. (1991) stocking rate has an impact on the quantity of the forage consumed and possibly the quality. Therefore, the objective of this research was to determine the difference in forage quality between grazed pastures vs. non-grazed pastures in the Nebraska Sandhills subirrigated meadows.

Materials and Methods

Under the approval of the University of Nebraska, Institutional Animal Care and Use Committee, esophageally fistulated cows were utilized to determine the effects of grazing on forage quality. A total of eight subirrigated meadow pastures (65ha ± 19 ha) in the Nebraska Sandhills were used. The meadow was divided into multiple pastures to allow rotational grazing. Of the eight sampled pastures, two adjacent pastures were sampled on one of four dates: early June, late June, early July, or late July. Of the two adjacent pastures sampled each date, one pasture was ungrazed while the other pasture had been grazed the previous 4 days with the exception of the late July pasture which was grazed for 3 days. On each sampling date the non-grazed pasture was sampled prior to grazing rotation and the grazed pasture was sampled after rotation to the non-grazed pasture had occurred. Stocking rates consisted of 37, 37, 74 and 47 animal unit days per ha for early June, late June, early July, and late July respectively. Because of severe drought, stocking rate was reduced in late July.

Three esophageally fistulated cows were used to sample each pasture on each date to determine forage quality. Esophageally fistulated animals have been used to collect diet samples used for analysis, as they provide a more accurate estimate of the forage that the animal is consuming (Weir and Torell, 1959). Prior to each diet sample collection, cows were withheld from feed, but not water, for 12 hr then transported to pastures where diets were to be collected. Cows were fitted with solid bottom bags after removal of the esophageal plug and introduced to the pasture then allowed to graze for about 20 min.

Immediately after collection, diet samples were frozen and stored at -20º C. Samples were lyophilized, ground to pass a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and analyzed for nitrogen using a Leco, FP 2000 combustion nitrogen analyzer (Leco Corp, St. Joseph, MO) then converted to CP by multiplying by 6.25. Neutral detergent fiber content was determined using the Van Soest et al. (1991) method, and IVDMD using the Tilley and Terry (1963) method with the modification of adding 1 g of urea to the buffer. Data were analyzed using the PROC MIXED procedure in SAS (SAS Inst., Inc., Cary, NC).

Results and Discussion

In early June CP ($P < 0.001$) and IVDMD ($P = 0.03$) content were greater and NDF ($P = 0.07$) content was lower in non-grazed compared with grazed pastures. Late June pastures exhibited similar patterns such that non-grazed pastures were greater in CP ($P = 0.03$) and tended to be greater in IVDMD ($P=0.09$) than grazed pastures. Late June NDF was numerically lower ($P=0.11$) for non-grazed compared with grazed pastures. The CP content of pastures in early July did not differ ($P = 0.30$) between non-grazed vs. grazed treatments. Neutral detergent fiber tended ($P=0.08$) to be lower and IVDMD tended ($P=0.09$) to be greater for non-grazed compared with grazed pastures in early July. With the higher stocking rate during this sampling period, it seems logical that this is when the greatest nutrient differences would have occurred. However, the results from our study could be due to the nature of the cool-season grass species being lower quality during July. Late July non-grazed pastures had greater ($P = 0.05$) CP levels than grazed pastures. However, there were no differences between non-grazed vs. grazed pastures for NDF ($P = 0.56$) or IVDMD ($P = 0.78$) in late July. These data suggest the greatest impact of grazing on forage quality occurs early in the grazing period. Lardy et al. (2004) also observed decreased CP content of diet samples collected from un-grazed subirrigated meadow pastures from June to July followed by an increase in CP levels from July to August. The later July sampling was higher than the early July sampling suggesting a similar trend. Severe drought during 2012 may have affected the quality of the July pastures. It also may be a possibility that as the season progressed and less water was present in the meadow the cattle would have been able to reach forage that they might not have been able to reach on an average precipitation year. More research is needed to determine the impact of stocking rate on forage quality both early and later in the growing season.

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Table 1. Crude protein, NDF and IVDMD values of masticate samples from Sandhills meadow

Item	Non-Grazed[1]	Fully Grazed[2]	SE	P-value
Early June[3]				
CP %	12.0	7.1	0.3	<0.001
NDF %	66.8	75.7	4.8	0.07
IVDMD %	66.7	58.1	2.3	0.03
Late June[3]				
CP %	9.8	7.2	0.3	0.03
NDF %	70.4	78.7	2.5	0.11
IVDMD %	63.5	57.0	1.4	0.09
Early July[3]				
CP %	8.7	8.3	0.2	0.30
NDF %	61.8	67.8	1.3	0.08
IVDMD %	58.2	54.1	1.4	0.09
Late July[3]				
CP %	10.0	8.0	0.5	0.05
NDF %	66.1	63.0	3.5	0.56
IVDMD %	54.6	54.0	1.4	0.78

[1] Pastures sampled prior to grazing
[2] Pastures sampled after grazing
[3] Date pasture was sampled using esophageally fistulated cows

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INFLUENCE OF BEEF CATTLE STOCKING DENSITY ON UTILIZATION OF VEGETATIVE COMMUNITIES IN A LATE-SPRING EARLY-SUMMER NATIVE BUNCHGRASS PRAIRIE.

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ABSTRACT: This study evaluated the influence of cattle stocking density on the use of different botanical communities on a native bunchgrass prairie. In each of two years, 192 cow-calf pairs (549.27 kg, BCS = 4.89) and 48 yearling heifers (383.34 kg, BCS = 5.02) were stratified by age and body condition, and randomly selected for a randomized block design (four blocks) with the following treatments: 1) Control, no livestock grazing; 2) low stocking, 0.36 animal units (AU)/ha; 3) moderate stocking, 0.72 AU/ha; and 4) high stocking, 1.08 AU/ha for a 42 d grazing period (late May to early July). Within each pasture, 36 monitoring sites were established uniformly along a grid of 6 north to south and 6 east to west transects. Using standing crop data from clippings at each monitoring site and relative preference data for cattle collected from the treatment area; the following 5 vegetation classifications were developed: 1) >20% introduced; 2) >40%native bunchgrass >50% Idaho fescue; 3) >40% native bunch grass <50% Idaho fescue; 4) >50% Forb; 5) Other. Utilization values were then collected at each monitoring site at the end of each grazing period. High stocking density had higher utilization values across all vegetation classifications ($P < 0.05$). At a pasture scale, moderate stocking density was over 11% more utilized than low, however, low and moderate stocking densities had no significant differences between vegetation classes 1 ($P = 0.172$, 39.57%), 2 ($P = 0.154$, 22.56%) and 4 ($P = 0.306$, 18.16%) whereas, vegetation classes 3 and 5 were utilized higher in the moderate stocking density than the low ($P <0.01$). In summary, there is a strong variation of grazing that occurs within a pasture containing differing vegetative communities. Due to grazing behavior and selectivity; stocking density may provide a very limited role when protecting a sensitive community type from over utilization.

Key Words: Beef cattle, stocking density, grazing behavior

Introduction

Ecological impacts due to gazing pressures from livestock are continuing to be topics of discussion and concern that are directing policy for public land use. According to Belsky and Blumenthal (1997), domestic livestock may alter ecosystem processes by reducing the cover of herbaceous plants and litter, disturbing and compacting soils, reducing water infiltration rates, and increasing soil erosion. Thus, suggesting that grazing plays an influential role in the ecological function of grassland communities. This makes it imperative for managers to understand grazing behavior and its application on the landscape.

In recent years there has been a strong effort to engineer grazing systems that will increase capacity and livestock performance (Walker 1995). Thus, many studies have been conducted testing different grazing systems and their potential effects on ecological condition as well as animal performance (Tracy and Faulkner 2006, Walburger et al. 2000, Pavlů et al. 2006, Holechek et al. 1987). Evidence generated from over 60 years of grazing experiments indicates that there is no true superior grazing system (Briske et al. 2008). However, it is generally accepted that selection of the correct stocking rate is the most important of all grazing management decisions (Holecheck et al. 2004). Effects of varying management such as stocking densities on plant species diversity may have important consequences for grassland community stability and ecosystem function (Hickman, et al. 2004).

Many management strategies such as grazing systems and stocking density are used to improve grazing distribution across the landscape specifically looking at optimizing animal performance and uniform utilization of vegetation. However, this does not take into account grazing selectivity and its impacts to vegetative communities. Relative to its impact on plant community structure, diet selection is by far the most important aspect of foraging behavior (Walker, 1995). With the selectivity of forage types being a main driver of grazing behavior, it is critical to look more in depth into the interaction of grazers and the utilization of vegetative communities within a paddock rather than the utilization of the paddock as a whole. With stocking density being one of the easiest management tools, when a preferred vegetative site becomes stressed from over grazing, managers tend to compensate by lowering the stocking density.

Our study was designed to test the effects of cattle stocking density on the utilization of vegetative communities found within a paddock. We hypothesize that although increased stocking densities may improve uniform utilization across a paddock; utilization values of preferred community types within the paddock are variable and influenced by palatability.

Materials and Methods

This study was conducted from late May to early July of 2007 and 2008 in northeast Oregon at The Nature Conservancy's Zumwalt Prairie Preserve. The Zumwalt Prairie is located on a basalt plateau at an elevation of 1340 to 1460 m with a mean slope of 7%. This area receives around 330 mm of precipitation annually falling in spring as localized thunderstorms and in winter as snow with a distinct dry period lasting from July through August. The average annual temperature is 6.4°C, and ranges from -2.8°C in December to 17.1°C in July (Damiran et al. 2007). Vegetation is dominated by native bunchgrass species that include Idaho fescue (Festuca idahoensis), prairie Junegrass (Koeleria macrantha), and bluebunch wheatgrass (Pseudoroegneria spicata) with the main forbs species consisting of prairie smoke (Geum triflorum), twin arnica (Arnica sororia) and lupin (Lupinus spp.). Shrub species make up a very small portion of the study area and consist of primarily snowberry (Symphoricarpos albus) and wild rose (Rosa spp.). Total mean annual production for vegetation across the study area ranges from 1262 to 1928 kg/ha (Darambazar et al. 2007).

This study was conducted as a randomized complete block design with four grazing treatments. The total study area consisted of 640 ha that was fenced into four 160 ha blocks each containing four 40 ha paddocks. The four grazing treatments (control, low, moderate, and high) were randomly assigned to each paddock within each block. Within each paddock, 36 monitoring sites were established uniformly along a grid of 6 north to south and 6 east to west transects using global positioning systems (GPS).

In each of the two years, 192 cow-calf pairs (549.27 kg, BCS = 4.89) and 48 yearling heifers (383.34 kg, BCS = 5.02) were stratified by age and body condition, and randomly assigned to the following treatments: 1) control, no livestock grazing; 2) low stocking, 0.36 animal units (AU)/ha; 3) moderate stocking, 0.72 AU/ha; and 4) high stocking, 1.08 AU/ha for a 42 day grazing period. Treatments were derived by setting the historic stocking densities for the Zumwalt Prairie as moderate, low was 50% of the moderate stocking density and high was 150% of the moderate stocking density.

Prior to the initiation of the grazing treatments standing crop data was collected by species at each of the monitoring sites in late spring to early summer of 2006. Relative preference values for cattle were then established during the grazing periods of 2007 and 2008 (Wyffels et al. 2009). The standing crop and relative preference values were then used to develop vegetation community criteria. Vegetation communities were then classified as follows: 1) >20% introduced; 2) >40% native bunchgrass >50% Idaho fescue; 3) >40% native bunch grass <50% Idaho fescue; 4) >50% Forb; and 5) Other. Idaho Fescue was used as a key species for breaking out native bunchgrass communities due to its prevalence, its correlation to ecological sites and its relative preference by cattle (Wyffels et al. 2009).

Post treatment utilization estimates were obtained at the monitoring sites by means of ocular estimates similar to that described by Parsons et al. (2003) using regression to adjust for observer error. Each community type within each stocking treatment was attributed a utilization value allowing for comparisons of use to be made across stocking densities.

Data was analyzed using proc mixed procedures of SAS (2002-2010). Least significant difference was used to separate means. Differences were considered significant at $P < 0.05$.

Results and Discussion

Control, non-grazed, paddocks had no differences in utilization values across vegetation classifications ($P > 0.05$) with an overall utilization 9.41%. Herbivory in the control paddocks are attributed to native herbivores, small mammals, and/or insects. In the low stocking density paddocks (average utilization of 20.97%) introduced vegetative communities (class 1) were utilized higher than all other vegetative classes ($P < 0.01$). All other vegetative communities (classes 2-5) showed no difference in levels of utilization ($P > 0.28$). Holechek (2004) noted that with light grazing only choice plants are used, whereas poor areas seem relatively undisturbed. In the moderate stocking density paddocks, average utilization of 30.5%, introduced vegetative communities (class 1) were grazed at higher levels than bunchgrass and forb vegetative communities (classes 2,3, and 4;$P < 0.01$) and tended to be utilized higher than non-classified vegetative communities (other, class 5, $P = 0.05$). Bunchgrass and forb vegetative communities (classes 2-4) showed no difference in levels of utilization ($P > 0.1$) with a tendency for non-classified vegetative communities (other, class 5) being utilized at higher levels than Idaho fescue dominated bunchgrass communities (class 4, $P = 0.08$). Non-classified vegetative communities were also utilized at higher levels than forb dominated communities (class 4, $P = 0.02$). High stocking density paddocks (average utilization of 46.01%) had higher utilization values across all vegetation classifications ($P < 0.05$). Introduced vegetative communities (class 1) had higher utilization rates than Idaho fescue dominated bunchgrass communities (class 2), forb dominated communities (class 4), and non-classified vegetative communities (class 5, $P < 0.008$) and tended to be grazed higher than bunchgrass communities lacking Idaho fescue (class 3, $P = 0.09$). Also in the high stocking density paddocks, bunchgrass communities lacking in Idaho fescue (class 3) had higher utilization values than Idaho fescue dominated bunchgrass communities (class 2, $P < 0.02$).

Moderate stocking density paddocks were around10% more utilized than low, however, low and moderate stocking densities had no significant differences between introduced vegetative communities (class 1, P=0.157, 39.57%), Idaho fescue dominated bunchgrass communities

(class 2, P=0.139, 22.56%) and forb dominated communities (class 4, P=0.278, 18.16%). Bunchgrass communities lacking in Idaho fescue (class 3) and non-classified vegetative communities (class 5) were utilized higher in the moderate stocking density than the low (P<0.002). There were no differences in vegetation class 4 between control and low stocking densities (P>0.09, 15.85%). Huber et al. (1995) has shown that stocking density did not alter botanical composition of the diet. This is reflected in the utilization rates of vegetation classes in our study with the exception that as stocking increases non-preferred vegetative communities become more utilized.

In our study, beef cattle forage utilization was variable within paddocks containing differing vegetative communities. In addition, at the light and moderate stocking rates, utilization patterns were strongly influenced by vegetation community preference. Only at the high stocking densities (150% of traditional use) did utilization patterns converge among plant communities. Therefore, due to grazing behavior and selectivity, stocking density may provide a very limited role when protecting preferred community types from over utilization.

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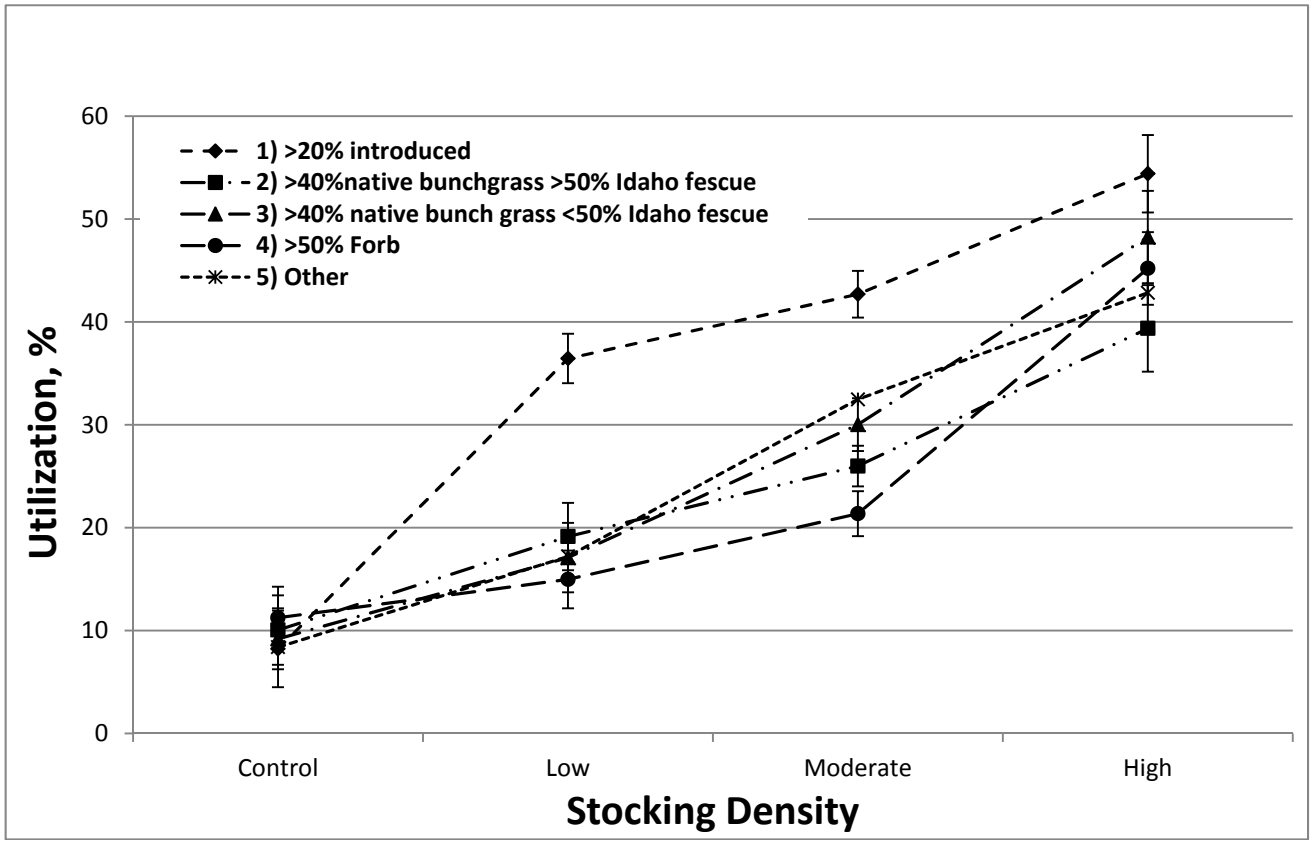

Figure 1. Differences in utilization of vegetative communities defined by plant community type and preference of beef cattle grazing at different stocking densities on the Zumwalt Prarrie in Northeast Oregon.

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THE INFLUENCE OF HABITAT TYPE ON VEGETATION QUALITY IN MIXED-CONIFER FORESTED RANGELANDS.

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ABSTRACT: We evaluated the influence of vegetation types within mountain Montane habitats of the inland Pacific Northwest. Specifically, we sampled vegetation by most common plant species for four grassland types (riparian grass, riparian sedge/rush, bunchgrass, and scabland) and six forest types that focused on early and late sucessional ponderosa pine, Douglas fir, and grand fir overstories. A minimum of 4 plots were established for each habitat type and vegetation was sampled in early-May, mid-June, early-August, and mid-September in 2004 and 2005. Approximately 2500 plant species forage quality samples were analyzed for CP, ADF, and NDF. All forage quality variables displayed a habitat type by sample date interaction ($P < 0.01$). Habitat types were similar ($P < 0.05$) in CP for the early-May and mid-June sampling dates averaging 18.6% and 11.3%, respectively. In contrast, the August sampling indicated that forested vegetation types were higher in CP than bunchgrass and scabland vegetation types ($P < 0.05$) with riparian grass and sedge/rush types being intermediate ($P > 0.10$). In addition, grand fir late succession vegetation types were higher in CP than all other habitats ($P < 0.01$). CP levels for the mid-September sampling period were highest in the Grand fir and Douglas fir vegetation types compared to riparian grass, bunchgrass and scabland vegetation types ($P < 0.05$) with Ponderosa pine and riparian sedge/rush vegetation types being intermediate ($P > 0.10$). Vegetation fiber constituents followed similar changes in composition with NDF and ADF increasing with early-August and mid-September sampling periods and difference between the forested vegetation and the non-forested vegetation types ($P < 0.05$). In summary, our results demonstrate a divergence in vegetation quality between forested and grassland vegetation types in late phenological stages of vegetation growth in mountain Montane habitats.

Key Words: Beef cattle, forage quality, vegetation type, forested rangelands

Introduction

Forested ecosystems present a broad array of existing and potential plant communities based both on plant associations (sensu Daubenmire 1952) and successional stage. These different combinations of plant associations and vegetation successional stages have been termed ecological land units in resource planning (Haufler et al. 1996). Forage quantity and quality, which theoretically vary across ecological land units, are important factors that affect habitat selection by ungulates. Managing forested ecosystems for sustainable and multiple uses requires that managers have a thorough understanding of how type and intensity of resource dynamics influences other ecosystem components. In particular, understanding how different silvicultural prescriptions affect future understory vegetation composition, production, and nutritive quality is essential to forest managers in order to maintain sustainable timber, livestock, and wild ungulate production. In addition, many of the forested rangelands throughout the western US also contain grassland habitat types that consist of various combinations of bunchgrass prairie and riparian meadows. Numerous researchers have observed variations in habitat use as influenced by habitat type within forested rangeland allotments (Parsons et al., 2003; DelCurto et al., 2005). Unfortunately, research designed to model secondary succession in forests affected by multiple disturbance agents is rare (Riggs et al., 2000). Likewise, research designed to describe the variation in forage quality and quantity among ecological land units (habitat types) throughout the summer grazing period is limited.

The purpose of this study was to characterize changes in forage quality during the growing season for ten habitat types common to the mixed conifer forest of the inland Pacific Northwest.

Materials and Methods

Two studies were conducted to assess forage quality and quantity on mixed conifer forest in the inland Pacific Northwest. In study 1, 54 ecological land units (habitat types) were sampled in the Blue Mountains Ecological Province for forage production (McNab and Evers, 1994). Twelve plots per habitat type were sampled for vegetation composition (Yost 2001). Total standing crop by species was sampled on four of the 12 plots per ecological land unit per year for three years. Plots to be sampled in any given year were caged prior to the onset of the growing season to insure that confounding herbivory effects did not occur prior to sampling. Caged plots were circular with a 0.75m radius and the entire plot was clipped by species. Sample collection occurred in July and August in each of 3 years within as narrow a time frame as possible to capture total standing crop of each species. Clipped samples were air dried on a wire rack in the field, dried to constant weight in a forced air oven (55^{o} C), and weighed to estimate dry-

matter production. Live and dead tissues were separated to prevent confounding by prior-year production. Results from Study 1 have been reported in an earlier manuscript but, was the foundation for Study 2.

In study 2, 10 habitat types were selected for forage quality determination over the grazing period. Specifically, forested habitats included **ponderosa pine** (*Pinus ponderosa*), **Douglas fir** (*Psudotsuga menziesii*), and g**rand fir** (*Abies grandis*) habitats in **early** or **late** seral stages. In addition, four non-forested habitats common in forested rangeland allotments were identified that included: 1) **riparian**, > 50% grass communities; 2) **wet meadow**, > 50% sedge/rush communities; 3) **grasslands**, bunchgrass communities; and 4) **scablands**, shallow soiled ridge tops. Forage quality was sampled over two years and was replicated at least four times for each habitat type. When possible, sampling location coincided with the placement of "peak production" caged plots and samples were taken near the caged plots within the habitat designation. Based on the first year of peak production estimates in Study 1, all palatable species (representing a minimum of 80% of the understory production) were sampled corresponding to early May, mid June, early August and mid September time periods. Vegetation diversity varied among habitat types and ranged from 5 to 18 individual plant species per habitat. Analysis of nutrient quality was conducted on individual plant species. The average quality of all species per habitat type were then averaged and analyzed for dry matter, crude protein, neutral detergent fiber, and acid detergent fiber by species (Goering and Van Soest, 1970). Acid detergent fiber content was used to calculate digestible dry matter content of each species (Rohweder et al., 1978). Data were analyzed using Proc Mixed with repeated measurescite SAS?in order to determine changes in forage quality over the grazing season. When habitat type interacted with sampling time ($p < .05$), data were sorted by time period and least significant differences were used to separate means ($p < .05$).

Results and Discussion

Crude protein displayed a habitat by sampling date interaction ($P < 0.05$; Figure 1). In early May, riparian habitats had the highest crude protein ($P < 0.05$) at 23.4% compared to all other habitats that averaged 18.1 % CP. For the mid June sampling date, all 10 habitats were similar in CP content averaging 11.3 % CP with the exception of the late seral grand fir habitats being higher in CP compared to scabland habitats ($P < 0.05$). In contrast, early August and late September sampling dates indicated the forested sites tended ($P = 0.08$) were higher in CP compared to the non-forested habitats. Forested habitats averaged 8.6 and 7.1 % CP whereas non-forested habitats averaged 6.8 and 6.0 % CP for the early August and mid September sampling periods, respectively. In addition, late seral grand fir sites were higher in CP that all non-forested and late seral ponderosa pine habitats ($P < 0.05$) during the early August sampling period. Likewise, in the early August sampling period, scablands had lower CP contents than all forested habitats.

A habitat x sampling date interaction was also observed for NDF and ADF ($P < 0.05$; Figure 2 and 3, respectively). In early May, all habitats had similar NDF and ADF contents ($P > 0.10$) averaging 43.2 and 25.7%, respectively. In contrast, forest communities were lower in NDF than non-forested communities for the mid June, early August, and mid September sampling periods ($P < 0.05$) with the exception of early seral grand fir habitats that did not differ from non-forested habitats ($P < 0.10$). In mid-June, early-August, and mid-September sampling dates, forested habitats (excluding early seral grand fir habitats) averaged 42.4 , 45.7, and 47.7% NDF, respectively compared to 53.5, 60.2, and 64.2% NDF, respectively in the non-forested plant communities. Acid detergent fiber followed the same pattern of differences except during the early August sampling period when early seral grand fir sites did not differ from wet meadows ($P < 0.10$). All forested habitats were lower in ADF compared to non-forested plant communities during the mid-September sampling period ($P < 0.05$) averaging 31.5 and 41.0%, respectively. In addition, early seral grand fir was higher in ADF than all other forested habitats ($P < 0.05$).

Mixed-conifer forest rangelands in the interior western U.S. are important forage resources for the western beef industry and provide critical habitat for wildlife species (Svejcar and Vavra, 1985). These areas are also diverse in respect to plant communities and present a challenge to sustainable rangeland management. This study suggests that these rangelands are high in quality and relatively uniform in quality early in the summer grazing period. However, as forages senesce due to a lack of moisture and/or plant communities reach mature phenological stages, forage quality diverges with substantial variation among vegetation habitats. Forested habitats are important in that they provide higher forage quality later into the summer grazing period compared to non-forested habitats. This observation is supported by other researchers who have documented the value of overstory characteristics on understory forage quality (Parsons et al., 2003; Walburger et al., 2007) and the shift in beef cattle distribution to forested north aspects late in the grazing period (DelCurto et al, 2005).

Implications

Forested rangelands in the inland Pacific Northwest are diverse with substantial variation in vegetation type because of large pasture sizes and dramatic differences in elevation and aspect. Our data suggests that these differences are minimal in early portions of the grazing period but become substantial when forage declines in value due to moisture stress and/or advancement of plant phenology. Forested vegetation types are critical in providing high quality nutrition for big game and livestock in the latter portions of summer and early fall.

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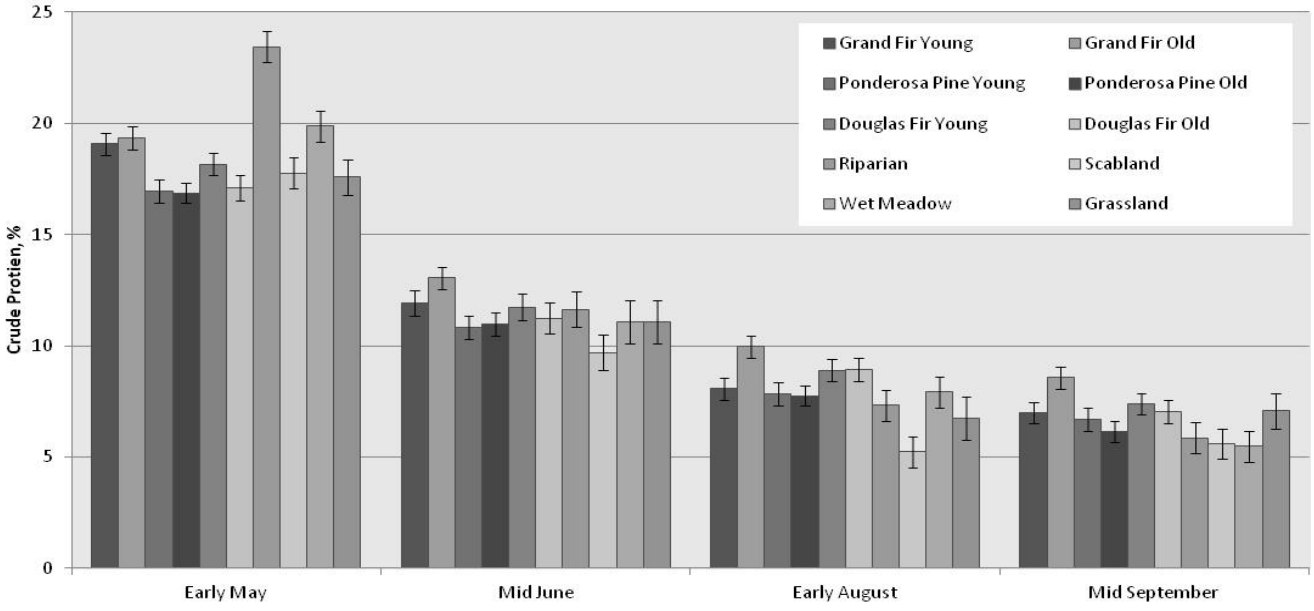

Figure 1. Crude Protein (CP) content for ten habitat types common to the mixed conifer forest of the inland Pacific Northwest across the spring, summer, and early fall grazing period. Crude Protein displayed a sampling time x habitat interaction ($P < .05$).

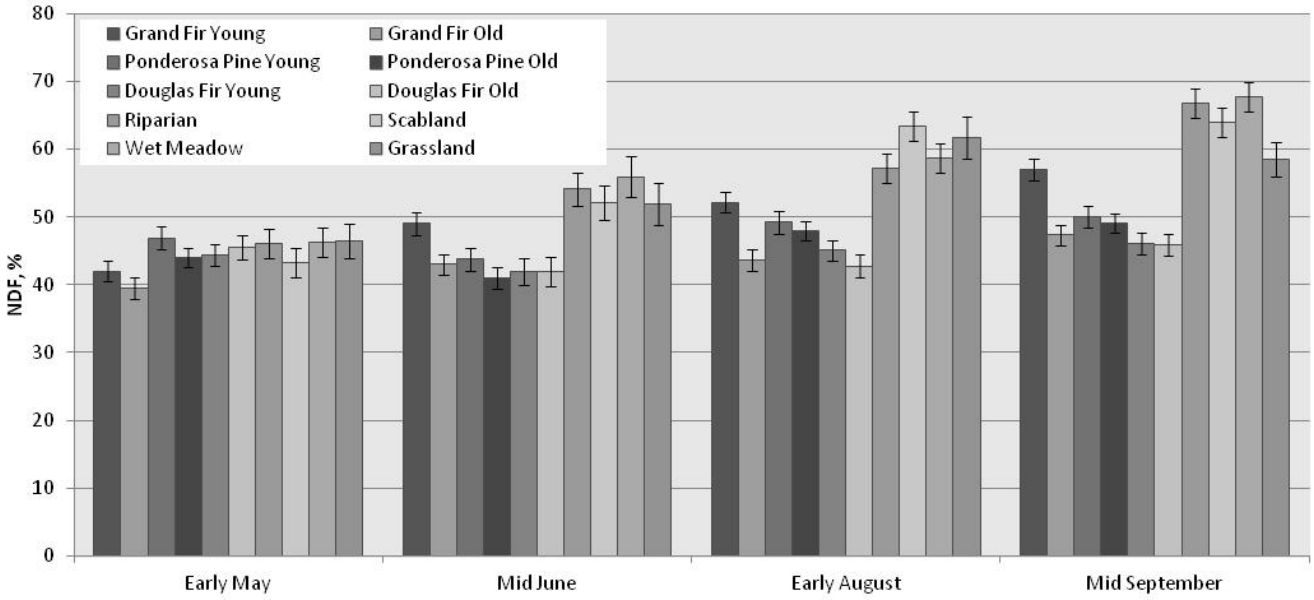

Figure 2. Neutral detergent fiber (NDF) content for ten habitat types common to the mixed conifer forest of the inland Pacific Northwest across the spring, summer, and early fall grazing period. Neutral detergent fiber displayed a sampling time x habitat interaction ($P < .05$).

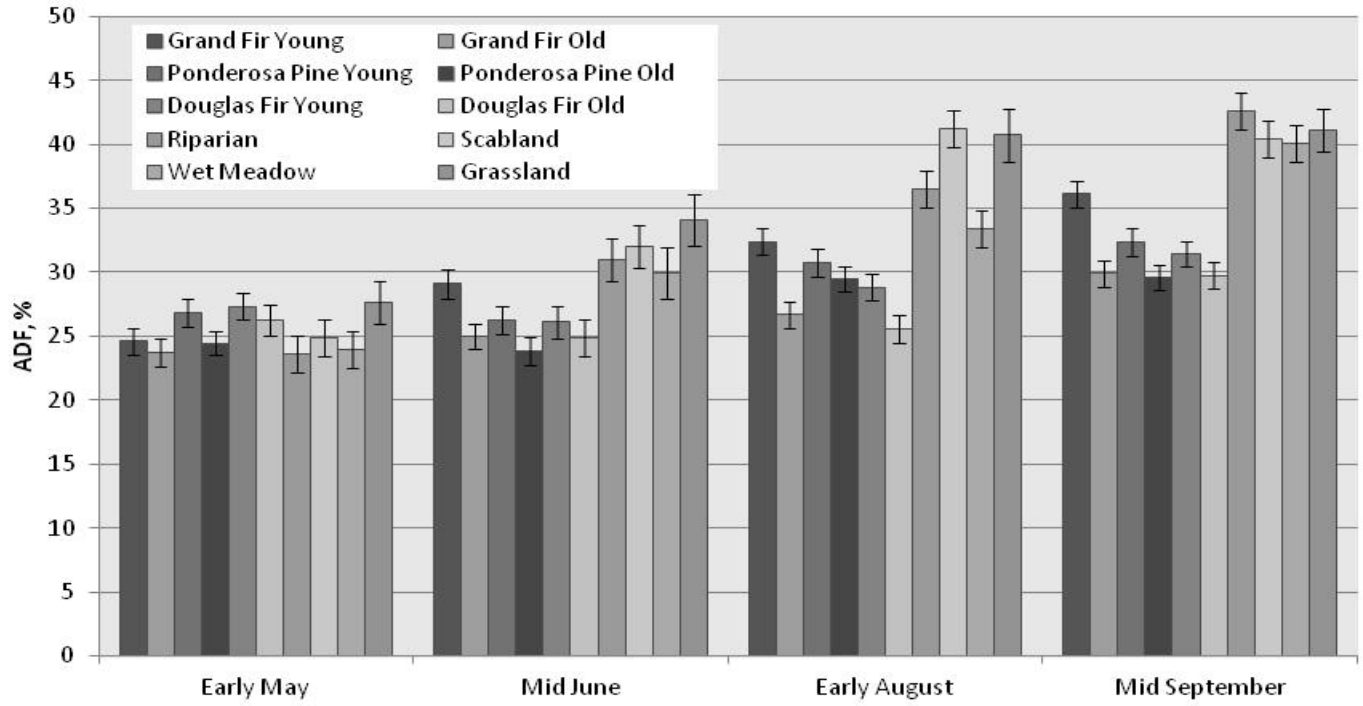

Figure 3. Acid detergent fiber (ADF) content for ten habitat types common to the mixed-conifer forest of the inland Pacific Northwest across the spring, summer, and early fall grazing period. Acid detergent fiber displayed a sampling time x habitat interaction ($P < .05$).

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THE INFLUENCE OF HABITAT TYPE AND OVERSTORY ON UNDERSTORY VEGETATION QUALITY IN MIXED-CONIFER FORESTED RANGELANDS.

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ABSTRACT: We evaluated the influence of habitat type and overstory characteristics on understory vegetation quality within Montane habitats of the inland Pacific Northwest. Specifically, we sampled vegetation by most common plant species for three forest types (ponderosa pine, Douglas fir, and grand fir stands), and, within each forest type, two successional stages (young versus mature stands), and two canopy closures (20 to 30% versus 40 to 60% canopy closures) which resulted in 12 unique plant community types. A minimum of 4 plots were established for each type and vegetation was sampled in early-May, mid-June, early-August, and mid-September in 2004 and 2005. Approximately 1750 understory plant species were analyzed for CP, ADF, and NDF. In respect to CP; habitat, canopy successional stage, and sampling date all displayed main effects ($p < .01$). However, we did observe a sampling date*habitat*successional stage interaction for CP ($p = .02$). In general, grand fir habitats were higher in CP compared to ponderosa pine ($p < .01$) with Douglas fir sites being intermediate ($p > .10$). In addition, late successional grand fir sites were higher in CP than all other forested habitats ($p < .05$). Understory NDF displayed a date*habitat*successional age interaction ($p < .01$). In general, understory NDF content was not influenced by stand age during early-May and mid-June sampling periods ($p > .10$), but, was reduced with older successional stages in the early-August and mid-September samples ($p < .05$). Understory vegetation ADF displayed a habitat*sampling date interaction ($p < .01$). In general, grand fir habitats were lower in ADF content at the early-May sampling period, but, highest for the mid-September sampling period as compared to Douglas fir and ponderosa pine habitats. The results of this study demonstrate the importance of overstory characteristics and stand type in determining the potential diet quality of understory vegetation.

Key Words: Beef cattle, forage quality, vegetation type, forested rangelands

Introduction

Forested ecosystems present a broad array of existing and potential plant communities based both on plant associations (sensu Daubenmire 1952) and successional stage. These different combinations of plant associations and vegetation successional stage have been termed as ecological land units in resource planning (Haufler et al. 1996). Forage quantity and quality, which theoretically vary across ecological land units, are important factors that affect habitat selection by ungulates. Managing forested ecosystems for sustainable and multiple uses requires that managers have a thorough understanding of how the type and intensity of resource dynamics influences other ecosystem components. In particular understanding how different silvicultural prescriptions affect future understory vegetation composition, production, and nutritive quality is essential to forest managers in order to maintain sustainable timber, livestock, and wild ungulate production. Numerous researchers have observed variations in habitat use as influenced by habitat type within forested rangeland allotments (Parsons et al., 2003; DelCurto et al., 2005). Unfortunately, research designed to model secondary succession in forests affected by multiple disturbance agents is rare (Riggs et al., 2000). Likewise, research designed to describe the variation in forage quality and quantity among ecological land units (plant communities) throughout the summer grazing period is limited.

The purpose of this study was to characterize changes in forage quality during the growing season of three forest types with different successional stages and different canopy closures.

Materials and Methods

We selected three forest types that included **ponderosa pine** (*Pinus ponderosa*), **Douglas Fir** (*Psudotsuga menziesii*), and **grand fir** (*Abies grandis*) habitats. In addition, we sub-divided each forest type into an **early** or **late** seral stage as well as a light (< 30% canopy cover) and moderate (> 40% but < 60%) canopy closure. Forage quality was sampled over two years and was replicated at least four times for each habitat type per year. All palatable species (representing a minimum of 80% of the understory production) were sampled corresponding to early-May, mid-June, early-August and mid-September time periods. Vegetation diversity varied among habitat types and ranged from 5 to 18 individual plant species per habitat. Analysis of nutrient quality was conducted on individual plant species. The average quality of all species per habitat type were than averaged and analyzed for dry matter, crude protein, neutral detergent fiber, and acid detergent fiber by species (Goering and Van Soest, 1970). Acid detergent fiber content was used to calculate digestible dry matter content of each species (Rohweder et al., 1978). Data were analyzed using Proc Mixed with repeated measures in order

to determine changes in forage quality over the late-spring, summer, and early-fall grazing season. When habitat type interacted with sampling time ($P < 0.05$), data were sorted by time period and least significant differences were used to separate means ($P < 0.05$).

Results and Discussion

Canopy cover did influence CP content of understory vegetation ($P < 0.05$) and tended to influence ADF content of understory forages ($P = 0.06$). In general, greater canopy closure resulted in lower CP and higher ADF compared to low canopy closures. As a result, canopy closure (shade) resulted in decreased quality of understory vegetation. Canopy closure, however, did not interact with habitat type, sampling time or successional age of forest stand ($P > 0.05$).

Crude protein displayed a sampling time x overstory type x sucessional stage interaction ($P < 0.05$). In early-May, grand fir habitats were higher ($P < 0.05$) than pondersoa pine habitats and late successional Douglas fir understories. Early seral Douglas fir understories being intermediate ($P > 0.10$). In midJune, late successional grand fir understories were higher in CP compared to ponderosa pine and Douglas fir understories ($p < 0.05$). For the early-August and mid-September sampling times, late successional grand fir understories were higher in CP than early successional grand fir, ponderosa pine, and Douglas fir understories ($P < 0.05$).

Like CP, NDF displayed a sampling time x overstory type x successional stage interaction ($P < 0.05$). In early-May, late successional grand fir understories were lower in NDF than ponderosa pine and Douglas fir ($P < 0.05$), but, not different from early successional grand fir ($P > 0.10$). In contrast, early successional grand fir understories were higher in NDF during the mid-June sampling period than all other forest types ($P < 0.05$) averaging 49.0% versus 42.8%, respectively. For the early-August sampling times, early successional grand fir was higher in NDF compared to late successional grand fir, late successional ponderosa pine, and Douglas fir understories ($P < 0.05$), but not different from early successional ponderosa pine ($P > 0.10$). In the mid-September sampling time, early successional grand fir understories were higher in NDF than all other habitat types ($P < 0.05$) averaging 56.9 versus 47.6%, respectively.

A forest type x sampling time interaction was observed for understory ADF ($P < 0.05$). In early-May, grand fir understories were lower in ADF ($P < 0.05$) than Douglas fir but not different from ponderosa pine understories ($P > 0.10$). In mid-June, all forest type understories were similar except grand fir tended to differ from ponderosa pine understories ($P = 0.06$). By the early-August sampling time, grand fir and ponderosa pine understories were higher in ADF than Douglas fir understories ($P < 0.05$). For the mid-September sampling time, grand fir understories were higher in ADF than Douglas fir ($P < 0.05$) with ponderosa pine understories being intermediate and not different ($P > 0.10$).

In general, forest types in the inland Pacific Northwest transition from ponderosa pine to Douglas fir, and Douglas fir to grand fir with increasing precipitation/moisture status. This habitat change can be a function of elevation, change in soil depth or aspect. Our results suggest that forest types are similar in forage quality across the season with modest changes in grand fir compared to ponderosa pine and Douglas fir understories. Previous research has also noted that grand fir and ponderosa pine habitats did not differ in dietary quality in August cattle diet collections (Walburger et al., 2007). In addition, our study found increased canopy closure correlated to understory quality with lower CP and higher ADF content. In contrast, Walburger and coworkers (2007) found no difference in beef cattle diet quality with non-logged forests versus thinned forests versus clear cut forested understories.

Implications

Forested rangelands in the inland Pacific Northwest are diverse with substantial variation in vegetation type because of large pasture sizes and dramatic differences in elevation, aspect, and soil depth. In our study, however, understory vegetation quality was similar across forest types with only slight changes in potential diet quality. Understory vegetation quality did decrease with forest canopy closure but did not seem to be dramatically altered by forest type or stand age. Furthermore, forested canopies are important in maintaining forage quality into late summer and early fall. Despite the lack of biological difference in forage quality among overstory habitat type, successional stage and canopy closure; forested habitats are important in maintaining late season understory quality for domestic livestock and wildlife.

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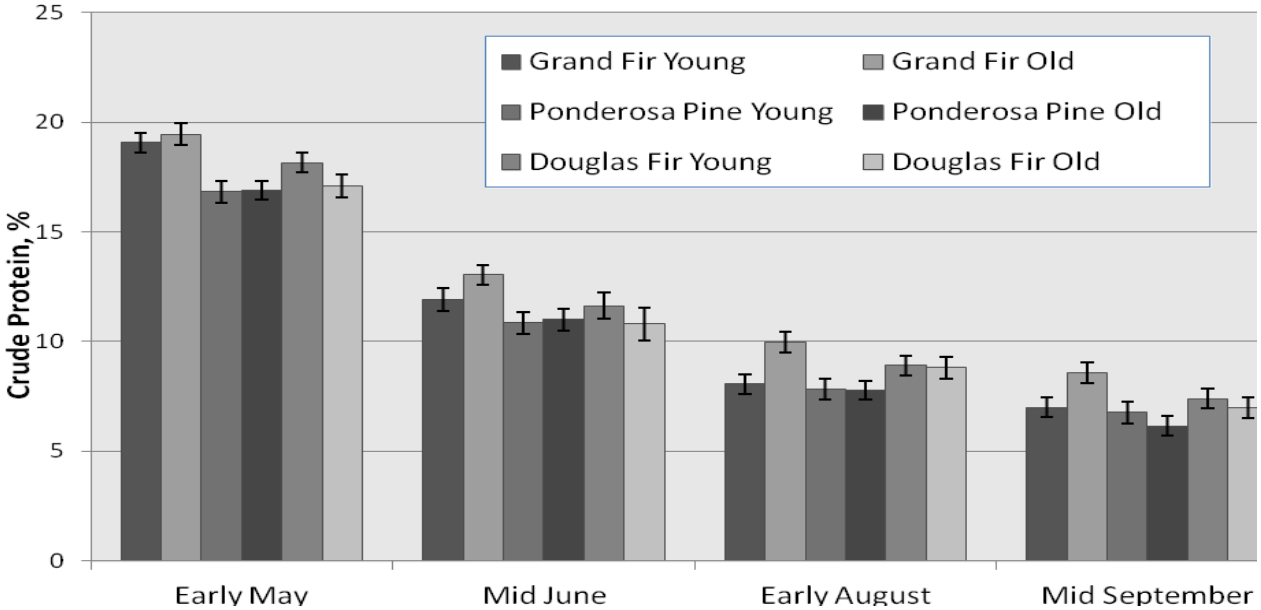

Figure 1. Crude Protein content during the growing season for three overstory types (ponderosa pine, Douglas fir, and grand fir) at two successional stages on forested rangelands in the inland Pacific Northwest. Crude Protein displayed a sampling time x overstory type x succession stage interaction ($P < .05$).

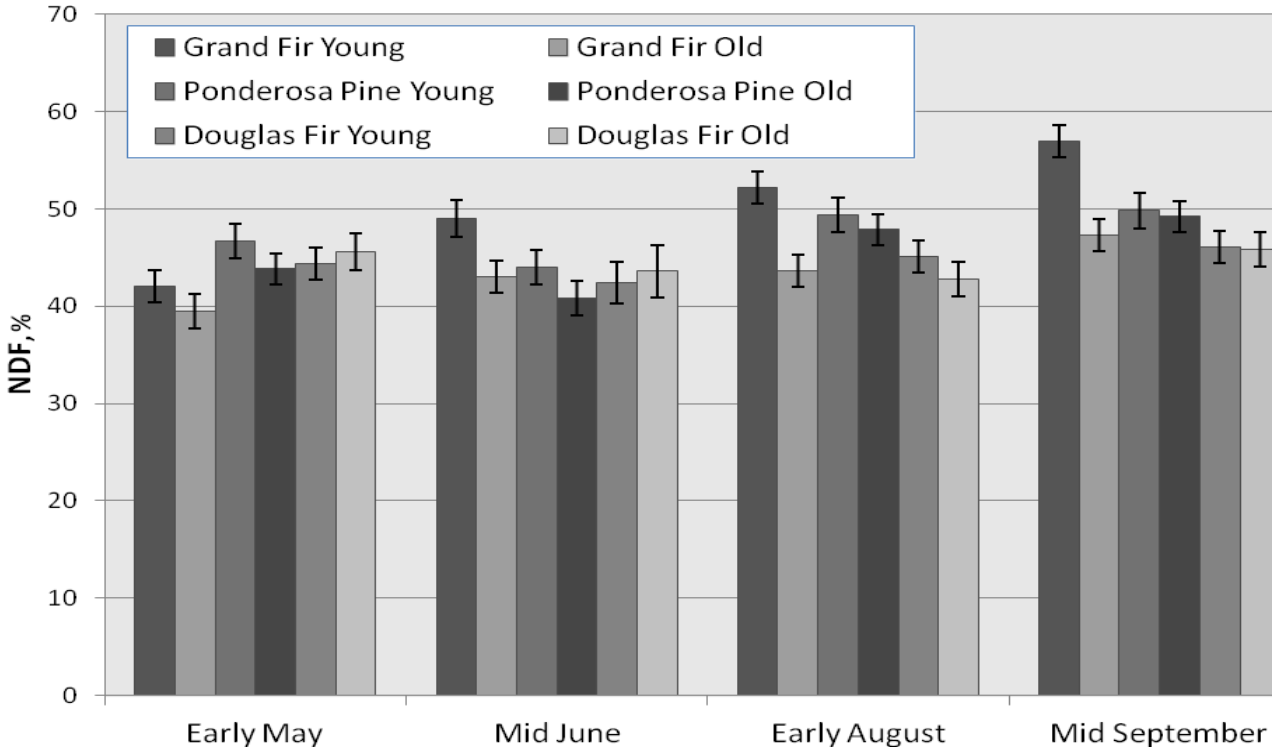

Figure 2. Neutral detergent fiber (NDF) content during the growing season for three overstory types (ponderosa pine, Douglas fir, and grand fir) at two successional stages on forested rangelands in the inland Pacific Northwest. Neutral detergent fiber displayed a sampling time x overstory type x succession stage interaction ($P < .05$).

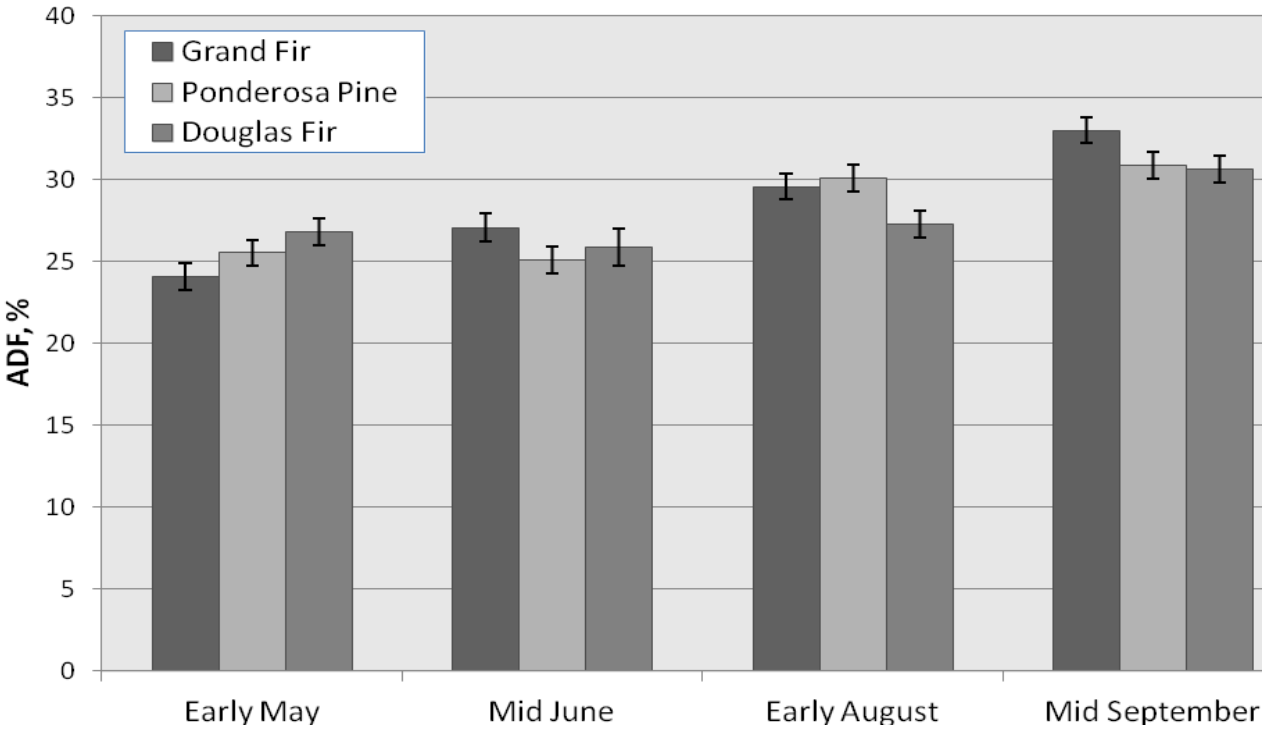

Figure 3. Acid detergent fiber (ADF) content during the growing season for three forest types (ponderosa pine, Douglas fir, and grand fir) common to the mixed conifer forest of the inland Pacific Northwest. Acid detergent fiber displayed a sampling time x forest type interaction ($P < .05$).

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GROWTH PERFORMANCE AND RUMINAL FERMENTATION CHARACTERISTICS OF GROWING BEEF STEERS FED BROWN MIDRIB CORN SIALGE-BASED DIET

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ABSTRACT: The objective of this study was to determine growth performance and ruminal fermentation characteristics of growing beef steers when fed a brown midrib corn silage-based TMR (BMRT) compared with a conventional corn silage-based TMR (CCST). This growing beef study was performed in a completely randomized design with 24 Angus crossbred steers (initial BW = 258 ± 23.2 kg) to test 2 treatments: CCST vs. BMRT. All animals were placed in individual pens, and 12 animals allocated to each treatment (n = 12). All steers were adapted to the CCST for a 2-wk period prior to start of the trial. The CCST contained 15.4% alfalfa hay, 48.1% conventional corn silage, 31.7% barley grain, and 5.3% feedlot supplement, whereas the BMRT consisted of 16.0% alfalfa hay, 49.0% brown midrib corn silage, 30.0% barley grain, and 5.0% feedlot supplement on a DM basis. All steers were fed once per day, and feed bunks read each afternoon and prior to morning feeding which was used to determine the amount of feed to deliver to each pen the following day. The experiment lasted 84 d. For all steers, BW and ruminal fermentation characteristics were measured on wk 4, 8, and 12. Intake of DM averaged 9.54 kg/d across the treatments and was similar between the treatments. Steers fed the BMRT tended to increase ADG compared to those fed the CCST (1.42 vs. 1.30 kg/d; $P = 0.08$). In addition, feeding the BMRT tended to increase G:F compared with the CCST (0.157 vs. 0.147; $P = 0.07$). Feeding the BMRT decreased ruminal pH (6.42 vs. 6.67; $P < 0.01$), whereas it increased total VFA concentration ($P = 0.01$) compared with the CCST. Feeding the BMRT decreased molar proportion of acetate, but increased propionate proportion, resulting in decreased acetate-to-propionate ratio compared with the CCST ($P < 0.02$). Overall data in this study indicate that feeding the BMRT to growing beef steers enhanced ruminal fermentation and shifted VFA profiles, which contributed to improved growth performance of steers fed the BMRT.

Key words: brown midrib corn silage, growing beef steers, ruminal fermentation

INTRODUCTION

Digestibility of forage fiber affects growth performance of rapid growing beef steers. In addition, providing adequate dietary concentrations of digestible fiber in cattle ration is essential for animal health, as it is required to support an appropriate rumen function. Typically, growing beef steers are fed forage-based diets, but lack of energy from forages and distention from rumen fill may limit DMI and reduce performance of high-producing dairy cows and rapidly growing beef steers (Holt et al., 2010; 2013). Therefore, great emphasis has been placed on dietary factors affecting DMI of cattle. The rumen-filling effect of diets is influenced most by concentration, digestibility, and fragility of forage NDF (Allen and Bradford, 2011). Feeding forages with enhanced digestibility of NDF has been reported to improve DMI and milk yield (Oba and Allen, 1999). Corn silage with the brown midrib mutation (**BMR**) has been well documented to have higher fiber degradability and will likely increase DMI and milk yield compared with cows fed conventional corn silage (**CCS**; Eastridge, 1999; Gencoglu et al., 2008). Therefore, energy sources high in digestible fiber, such as BMR, may allow for increased energy intake without disruption of fiber digestion and improved ruminal fermentation, which can enhance growth performance of growing beef steers. The objectives of this study were to determine the influence of feeding BMR-based diet to growing beef steers on the followings: 1) growth and feed intake, and 2) ruminal fermentation characteristics in growing beef cattle.

MATERIALS AND METHODS

The beef steers used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University, Logan.

Animals, Experimental Design, and Diets. A feedlot experiment was conducted at the Animal Science Farm at Utah State University, from October, 2012 to January, 2013. Twenty-four Angus crossbred steers (258 ± 23.2 kg) were randomly assigned into one of 2 dietary treatments: CCS-based TMR (**CCST**) and BMR-based TMR (**BMRT**). The 2 treatments were assigned to 12 steers each housed in individual pens in a completely randomized design, resulting in 12 replications per treatment. The CCST contained 15.4% alfalfa hay, 48.1% CCS, 31.7% barley grain, and 5.3% feedlot supplement, whereas the BMRT consisted of 16.0% alfalfa hay, 49.0% BMR, 30.0% barley grain, and 5.0% feedlot supplement on a DM basis (Table 1). The 2 dietary treatments had similar concentrations of CP, NDF, and ADF.

All steers were fed once per day, and each feed bunk was read each afternoon prior to the morning feeding. This was used to determine the amount of feed that needed to be delivered to each pen the following day. All steers had ad libitum access to fresh water. All steers were weighed twice every 4 wk to determine BW.

Two corn silage hybrids, conventional (Pioneer 9714; Pioneer Hi-breed International, Inc., Johnston, IA) and BMR (Mycogen Seeds, Indianapolis, IN) were planted in spring 2011. The harvested corn silage was ensiled

separately in bag silos (Ag/Bag International Ltd, Warrenton, OR).

Sampling, Data Collection, and Chemical Analyses. Samples of the TMR fed and orts for individual steers were collected weekly, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored for subsequent analyses. Contents of DM of the samples were used to calculate DMI. Analytical DM concentration of samples was determined by oven drying at 135°C for 3 h; OM was determined by ashing, and N concentration was determined using an elemental analyzer (Flash 2000 N/Protein Analyzer, Thermo Scientific, Cambridge, UK) (AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company. Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

Ruminal Fermentation Characteristics. Ruminal fluid samples were obtained using an oral stomach tube (Geishauser, 1993) 3 hours after morning feeding on wk 4, 8, and 12. The pH of the ruminal fluid was measured within 5 minutes of collecting the samples using a portable pH meter (Oakton pH6; Oakton Instruments, Vernon Hills, IL). Five mL of ruminal fluid were frozen and stored at –40°C for VFA analysis. Ruminal VFA were separated and quantified using a GLC (model 6890 series II; Hewlett-Packard Co., Avondale, PA) with a capillary column (30 m × 0.32 mm i.d., 1-μm phase thickness, Zebron ZB-FAAP; Penomenet Inc., Torrance, CA) and flame-ionized detection. The oven temperature was held at 170°C for 4 min, increased to 185°C at a rate of 5°C/min, then increased by 3°C/min to 220°C, and held at this temperature for 1 min. The injector and the detector temperatures were 225 and 250°C, respectively, and the carrier gas was helium (Eun and Beauchemin, 2007).

Table 1. Ingredients and chemical composition of beef steer diets fed in growing period (DM basis)

	Diet[1]	
Item	CCST	BMRT
Ingredient		
Alfalfa hay, chopped	15.4	16.0
Conventional corn silage	48.1	-
Brown midrib corn silage	-	49.0
Barley grain, dry rolled	31.7	30.0
Feedlot supplement[2]	5.3	5.0
Chemical composition		
DM, %	45.7	50.0
CP	10.2	10.6
NDF	35.1	32.7
ADF	18.0	17.3

[1]CCST = conventional corn silage-based TMR; BMRT = brown midrib corn silage-based TMR.

[2]Composition: 5.0% NaCl, 0.24% Mg, 0.76% K, 200 ppm Cu, 400 ppm Mn, 650 ppm Zn, 2 ppm Se, 22 ppm I, 9 ppm Co, 121,000 IU/kg Vitamin A, 37,400 IU/kg Vitamin D, 55 IU/kg vitamin E, and 360 ppm Rumensin® (Elanco Animal Health, Indianapolis, IN).

Statistical Analysis. All data in this study were analyzed using the MIXED procedure of SAS (SAS Institute, 2011). Animal was an experimental unit with monthly data collection periods as repeated measures of treatments. Data were analyzed using the following model: $Yijk = \mu + T_i + P_j(T)_i + M_k + TM_{ik} + \varepsilon_{ijk}$ where, μ = overall mean, T_i = fixed effect of dietary treatment i, $P_j(T)_i$ = random effect of animal j within dietary treatment i, M_k = effect of sampling month k, TM_{ik} = interaction between dietary treatment i and sampling month k, and ε_{ijk} = residual error. Because interactions were lacking in all cases, data were reanalyzed using a model that included treatment as a fixed effect and the random effect of animal, with months as repeated measures of the treatments. Simple, autoregressive one, and compound symmetry covariance structures were used in the analysis depending on low values for the Akaike's information criteria and Schwartz's Bayesian criterion. Significant effects of the treatment were declared if $P < 0.05$, and trends were accepted if $0.05 < P < 0.10$.

Table 2. Effect of feeding different corn silage hybrids to growing beef steers on growth performance and ruminal fermentation characteristics

	Treatment[1]			
Item	CCST	BMRT	SEM	*P*
BW				
Initial, kg	261	253	6.7	0.44
Final, kg	380	383	9.1	0.85
Change, kg	119	129	4.3	0.12
ADG, kg/d	1.30	1.42	0.047	0.08
DMI, kg/d	9.72	9.35	0.296	0.38
G:F	0.147	0.157	0.0041	0.07
Ruminal pH	6.67	6.42	0.034	< 0.01
Total VFA, m*M*	80.8	89.7	2.17	0.01
Individual VFA[2]				
Acetate (A)	64.9	60.5	0.689	< 0.01
Propionate (P)	18.7	21.8	0.703	0.01
Butyrate	9.52	12.3	0.369	< 0.01
A:P	3.39	2.75	0.136	< 0.01

[1]CCST = conventional corn silage-based TMR; BMRT = brown midrib corn silage-based TMR.

[2]Individual VFA expressed as mol/100 mol.

RESULTS

Dietary treatments did not affect BW change (Table 2). Intake of DM averaged 9.54 kg/d across the treatments and was similar between the treatments. Steers fed the BMRT tended to increase ADG compared to those fed the CCST (1.42 vs. 1.30 kg/d; $P = 0.08$). In addition, feeding the BMRT tended to increase G:F compared with the CCST (0.157 vs. 0.147; $P = 0.07$). Feeding the BMRT decreased ruminal pH (6.42 vs. 6.67), whereas it increased total VFA concentration compared with the CCST. Feeding the BMRT decreased molar proportion of acetate, but increased propionate proportion, resulting in decreased acetate-to-propionate ratio compared with the CCST. In addition,

feeding the BMRT increased molar proportion of butyrate compared with the CCST.

DISCUSSION

Retention of digesta in the rumen functions to supply a more consistent flow of nutrients to the small intestine, but physical fill of the gastrointestinal tract can limit feed intake, when high forage diets are fed. Feeding cattle BMR with increased NDF degradability has been shown to increase DMI. Keith et al. (1981) observed an increase in DMI (0.47 kg/d) with steers fed BMR. Tjardes et al. (2000) also observed an increase in DMI when feeding BMR (0.43 kg/d), but G:F decreased (0.145 vs. 0.135) with no effect on ADG (1.02 vs. 1.01 kg/d). In the current study, we did not observe an effect of BMR on DMI. New plant genetics in both fiber and starch for the BMR hybrid used in this study may have affected ruminal digestion kinetics, which may determine the site and extent of nutrient digestion and can greatly affect the type and pattern of fuels absorbed over time, thereby influencing the temporal pattern of fuel oxidation in the liver and feeding behavior. In the current study, feeding the BMRT increased VFA concentration, and favorably shifted ruminal fermentation pathway by increasing propionate proportion but decreasing acetate proportion. Besides increasing concentration of VFA produced per kg of OM fermented, increasing propionate as a proportion of VFA absorbed may cause a signal to terminate meals because propionate flux to the liver may increase greatly during meals (Benson et al., 2002), and is rapidly metabolized in the liver (Reynolds, 1995) which can stimulate hepatic oxidation. Allen et al. (2009) reported that VFA rapidly produced and absorbed during meals are likely responsible for stimulating satiety in ruminants. Holt et al. (2013) showed that feeding BMR to lactating dairy cows in the first 60 d of lactation had no effect on DMI, but reported a tendency to increase BW with feeding BMR (P = 0.09). This can be explained by the more energy available to the animal when feeding BMR.

Increased VFA concentration due to feeding the BMRT in this study supports increased energy supply for growth. The propionate from feeding the BMRT resulted in decreased ruminal pH, but feeding the BMRT would not interfere with ruminal fermentation, as average ruminal pH of the BMRT was 6.42. This shift in VFA proportions indicates a potential increase in availability of glucogenic precursors to animals consuming BMR. The shift in precursor availability could improve nutrient utilization, particularly for rapidly growing beef steers. Therefore, responses to feeding BMR may be highest in situations where fiber digestion and fermentation are major contributors to net energy supply, which is often the case for growing beef steers.

IMPLICATIONS

Our overall findings indicate that feeding the BMRT increased growth performance of growing beef steers due to enhanced ruminal fermentation and beneficial shifts of fermentation pathway. Knowledge gained from additional research will be necessary to understand physiological effects of feeding BMR to growing beef steers. In addition, it needs to be elucidated whether feeding BMR to beef steers in finishing phase will have similar effects on growth performance and ruminal fermentation profiles as were observed in growing phase.

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EFFECT OF FIBROZYME ON IN VITRO DIGESTIBILITY OF ALFALFA HAY MANIPULATING MATURITY STAGE AND MEDIUM pH

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ABSTRACT. Effects of an exogenous fibrolytic enzyme and incubation medium pH on *in vitro* digestibility of alfalfa hay cut at early and late bud, and early bloom stages were evaluated. Enzyme was applied to the incubation medium at levels of 0.0 (0), 0.0138 (1) and 0.0276 (2) g/g forage DM. Incubation medium pH was adjusted to an initial pH of 6.74, 6.15, and 5.39. Chemical composition of alfalfa and *in vitro* digestibility at 48 h were evaluated using a complete random design and a complete random design with a 3x3x3 factorial arrangement, respectively. Chemical composition was similar (P>0.05) between early and late bud stage of alfalfa. However, contents of ADF (24.9 vs. 20.0 and 22.4 %), cellulose (20.0 vs. 17.5 and 18.5 %) and lignin (4.96 vs. 3.65 and 3.81 %) were higher (P<0.01), and CP (19.6 vs. 23.5 and 23.3 %) was lower (P<0.01) in alfalfa cut at early bloom vs. early and late bud stages, respectively. Maturity×pH×enzyme interaction was significant (P<0.01) for *in vitro* digestibility of alfalfa. Enzyme did not increase digestibility at any maturity stage of alfalfa when medium pH was 6.74. At medium pH of 6.15 level 2 of enzyme vs. 0 improved (P<0.02) DM, NDF, ADF, hemicellulose and cellulose *in vitro* digestibility in late bud stage of alfalfa (84.6 vs. 83.1 %; 59.6 vs. 55.6 %; 48.6 vs. 44.4 %; 75.2 vs. 71.7 % and 58.2 vs. 47.5 %, respectively). At medium pH of 5.39 level 2 of enzyme vs. 0 improved (P<0.02) DM, NDF, ADF, hemicellulose and cellulose *in vitro* digestibility in early bloom stage of alfalfa (79.4 vs. 77.4 %; 48.4 vs. 43.4 %; 40.0 vs. 33.0 %; 62.2 vs. 60.6 % and 46.5 vs. 39.7 %, respectively). *In vitro* digestibility of hemicellulose was improved by level 1 of enzyme at medium pH of 5.39 in early and late bud, and early bloom stages of alfalfa (73.6 vs. 71.3 %, 74.4 vs. 71.6 % and 64.6 vs. 60.6 %, respectively). It is concluded that under low pH conditions (5.39) the use of fibrolytic enzyme improved hemicellulose *in vitro* digestibility regardless maturity state of alfalfa, as well as DM, NDF, ADF, hemicellulose and cellulose digestibility of early bloom alfalfa.

Key words: alfalfa hay, exogenous enzyme, maturity stage, pH

Introduction

Forage cell wall components are an energy source for ruminants through microbial rumen fermentation. However, fiber digestibility is limited by lignification (Akin, 1989).

Several strategies have been utilized to improve forage digestibility, including forage breeding programs (Beauchemin *et al.,* 2003). Recently, the use of exogenous fibrolytic enzymes has been renovated by they potential to hydrolyze cellulose and hemicellulose in synergy with microbial rumen enzymes (Elwakeel *et al.,* 2007), particularly under rumen pH lower than 6.0 (Colombato *et al.,* 2007).

Forage maturity and nutritional quality are negatively correlated, decreasing DM digestibility and animal performance (Lacefield, 2004). However, the effects of forage maturity and rumen pH on efficiency of exogenous fibrolytic enzymes have been poorly studied.

The objective of this study was to evaluate the impact of three levels of an exogenous fibrolytic enzyme on in vitro digestibility of dry matter and fiber components of alfalfa hay at three maturity stages and three medium pH conditions.

Materials and Methods

From a cultivar of Dairy Tall Green variety of alfalfa, forage samples were taken on November 6th, 15th and 30th of 2010, corresponding to early and late bud, and early bloom stages, respectively (Kalu and Fick, 1981).

The exogenous fibrolytic enzyme used was Fibrozyme® (Alltech Inc., Nicholasville, KY). The enzyme was added to the Daisy II incubator (ANKOM Technology Corporation., Fairport, NY, USA) in doses of 0.0 (0), 0.0138 (1) and 0.0276 (2) g/g of forage DM, respectively (Domínguez *et al.,* 2010).

Alfalfa samples were dried in a forced air oven at 60 ºC during 48 h and ground to 1 mm in a Wiley mill (A.H. Thomas, Philadelphia, PA, USA) to determine absolute dry matter and CP (AOAC, 2000). Content of NDF, ADF (Van Soest *et al.,* 1991), and ADL (Goering and Van Soest, 1970) were sequentially determined in the ANKOM^{200} Fiber Analyzer (Ankom Technology Corporation, Macedon, NY, USA) using Ankom® filter bags F57.

In vitro digestibility of alfalfa was done in the Daisy II Incubator (Ankom Technology Corporation, Macedon, NY, USA). Alfalfa samples (0.5 g ± 0.05) placed on Ankom® filter F57 bags were incubated during 48 h through three digestion runs using only three jars per run. Each digestion run evaluated different medium pH: 6.74, 6.15 and 5.39. In each run, every jar has different enzyme level (0, 1 and 2) and 7 bags were placed from each alfalfa maturity stage and 4 blank bags. Medium pH at incubation times (6.74, 6.15 and 5.39) were achieved by adding citric acid 1N in amounts of 0, 18 and 36 ml, respectively (Grant and Mertens, 1992). Medium and inoculum were prepared according to the *in vitro* true digestibility method of ANKOM®. The inoculum was produced with rumen fluid

and a handful of fibrous rumen mat obtained from the ventral and caudal sacs of rumen from two dairy cows through rumen canula.

Chemical composition and i*n vitro* digestibility of alfalfa were analyzed using a complete random design, and a complete random design with a 3x3x3 factorial arrangement, respectively, using the GLM procedure of SAS (SAS, 2005).

Results and Discussion

Chemical composition of alfalfa (Table 1) was similar at early and late bud stages. However, ADF, cellulose and ADL content were 17.4, 11.1 and 32.9%, higher, respectively (P< 0.05) in alfalfa at early bloom compared to early and late bud stages, meanwhile CP content was reduced by 16.2% (P<0.05). Cold weather observed during the 10 d of interval between early and bud stage of alfalfa probably could limit plant growth allowing similar chemical composition. The reduction of nutritional quality of alfalfa cut at early bloom stage has been related to the advanced cellular wall development (Hall et al., 2000).

An interaction (P<0.01) maturity×pH×enzyme was found for IVDMD, *in vitro* digestibility of NDF **(IVNDFD)**, *in vitro* digestibility of ADF **(IVADFD)**, *in vitro* digestibility of hemicellulose **(IVHEMD)** and *in vitro* digestibility of cellulose **(IVCELD)** of alfalfa.

A reduction (P<0.001) on IVDMD was observed as maturity stage of alfalfa advanced, being similar between early and late bud stage (83.9%), but lower at early bloom stage (78.7%). At medium pH of 6.74 and 6.15 IVDMD was similar (P>0.05), but it was higher (P<0.001) than at medium pH of 5.39 (82.3, 82.4 and 81.7%, respectively). This response has been associated to a lower fiber digestibility when medium pH is equal or lower than 6.0 (Hoover, 1986).

The addition of fibrolytic enzyme did not improve IVDMD of alfalfa (Table 2) at early bud stage, regardless medium pH. However, level 2 of enzyme vs. 0 and 1 increased (P<0.05) IVDMD of alfalfa at late bud stage (84.6 vs. 83.1 and 83.2%, respectively) when medium pH was 6.15, and at early bloom stage (79.4 vs. 77.4 and 78.3%, respectively) when medium pH was 5.39. Improvement on IVDMD of alfalfa has been reported by using a fibrolytic enzyme at medium pH of 6.2, but not at pH of 5.72 (Colombatto *et al.,* 2007).

Alfalfa hay cut at early and late bud stages showed higher (P<0.001) IVNDFD and IVADFD compared to early bloom stage (58.1 and 56.9 vs. 46.5%; 45.4 and 43.1 vs. 36.7%, respectively). At medium pH of 6.74 and 6.15 IVNDFD and IVADFD were similar (P>0.05), but higher (P<0.001) than at medium pH of 5.39 (54.4 and 54.5 vs. 52.7%; 41.9 and 43.1 vs. 40.3%, respectively). Lower pH (≤ 6.0) decrease fiber digestibility, since activity of celulolytic microorganisms is depressed (Shriver *et al.,* 1986).

The use of enzyme did not improve IVNDFD and IVADFD of alfalfa at early bud stage at any medium pH (Table 2). Adding the enzyme at level 2 compared to 0 and 1 at medium pH of 6.15 improved IVNDFD (P<0.001) of alfalfa at late bud and early bloom stages (59.6 vs. 55.6 and 56.0%; 48.4 vs. 43.4 and 45.6% respectively).

An improvement (P<0.05) in IVADFD of alfalfa at late bud stage was achieved by level 2 of fibrolytic enzyme vs. 0 when medium pH was 6.15 (48.6 vs. 44.4%). Addition of level 2 of enzyme vs. 0 and 1 increased IVADFD of alfalfa at early bloom stage when medium pH was 5.39 (40.0 vs. 33.0 and 34.1%, respectively).

In contrast, Colombatto *et al.* (2007) found an increase in IVNDFD and IVADFD of alfalfa at 24 h of incubation, when a fibrolytic enzyme was added at medium pH of 6.5 and 6.72, but not at medium pH of 5.72 and 6.2

Alfalfa hay cut at early and late bud stages showed higher (P<0.001) IVHEMD and IVCELD than alfalfa at early bloom stage (73.3 and 73.3 vs. 62.9%; 54.0 and 52.3 vs. 44.2%, respectively). At medium pH of 6.74 and 6.15 IVHEMD and IVCELD were similar (P>0.05) but higher (P<0.05) than a medium pH of 5.39 (70.9 and 69.6 vs. 69.0%; 51.8 and 51.3 vs. 47.3%, respectively).

No advantage on IVHEMD by use of fibrolytic enzyme was observed for alfalfa at early bud stage when medium pH was 6.74 and 6.15 (Table 3). However, IVHEMD at early and late bud, and early bloom stages of alfalfa was improved (P<0.05) by level 1 of enzyme vs. 0 at medium pH of 5.39 (73.6 vs. 71.3%; 74.4 vs. 71.6%, 64.6 vs. 60.6%, respectively). Alfalfa at late bud stage showed a higher IVHEMD by use of level 2 of enzyme vs. 0 and 1 (75.2 vs. 71.7 and 72.0%, respectively) at medium pH of 6.15. In agreement, Colombatto *et al.* (2007) found higher IVHEMD of alfalfa when adding a fibrolytic enzyme under medium pH of 5.72.

Improvements (P<0.05) of 17.5% on IVCELD at late bud stage of alfalfa were observed by level 1 and 2 of fibrolytic enzyme vs. 0 when medium pH was 6.15 (53.4 and 58.2 vs. 47.5%, respectively). Similarly, level 2 of enzyme vs. 0 increased by 17.1% (P<0.05) IVCELD at medium pH of 5.39 (46.5 vs. 39.7%).

Conclusions

The highest benefits on fiber digestion were achieved using level 2 of fibrolytic enzyme in alfalfa at early bloom stage when medium pH was 5.39.

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Table 1. Effect of maturity stage on chemical composition (% DM) of alfalfa hay.

	Maturity stage		
Item	Early bud	Late bud	Early bloom
NDF	37.7 ± 1.4	38.2 ± 2.0	39.9 ± 1.4
ADF	20.0 ± 0.6^{b}	22.4 ± 0.6^{b}	24.9 ± 0.6^{a}
Hemicellulose	16.6 ± 1.3	15.8 ± 1.9	14.9 ± 1.3
Cellulose	17.5 ± 0.1^{c}	18.5 ± 0.1^{b}	20.0 ± 0.1^{a}
CP	23.5 ± 0.5^{a}	23.3 ± 0.5^{a}	19.6 ± 0.5^{b}
ADL	3.65 ± 0.1^{b}	3.81 ± 0.1^{b}	4.96 ± 0.1^{a}

Table 2. Effect of maturity stage of alfalfa, medium pH and level of fibrolytic enzyme on *in vitro* digestibility of DM, NDF and ADF.

		Enzyme[1]								
		0			1			2		
Maturity stage	Medium pH	MS	NDF	ADF	MS	NDF	ADF	MS	NDF	ADF
Early bud	6.74	85.1^{a}	60.4^{a}	46.7^{a}	83.7^{b}	56.9^{b}	39.6^{b}	84.6^{ab}	59.2^{ab}	43.4^{ab}
	6.15	85.2	60.8	47.0	84.4	58.7	45.0	84.3	58.4	44.1
	5.39	83.3	55.8	40.5	83.4	56.1	39.2	83.7	56.7	42.4
Late bud	6.74	83.9	57.8	46.5	83.3	56.2	43.8	84.3	59.0	47.7
	6.15	83.1^{b}	55.6^{b}	44.4^{b}	83.2	56.0^{b}	44.8^{ab}	84.6^{a}	59.6^{a}	48.6^{a}
	5.39	83.0	55.4	43.9	83.8	57.6	45.8	82.9	55.1	43.4
Early bloom	6.74	80.0^{a}	49.8^{a}	41.2^{a}	78.3^{b}	45.6^{b}	35.4^{b}	77.9^{b}	44.5^{b}	32.6^{b}
	6.15	79.2	47.9	38.6	78.8	46.8	38.5	78.9	47.0	37.1
	5.39	77.4^{b}	43.4^{b}	33.0^{c}	78.3^{b}	45.6^{b}	34.1^{b}	79.4^{a}	48.4^{a}	40.0^{a}

[1] 0= 0.0, 1= 0.0138 and 2= 0.0276 g of Fibrozyme®/g of forage DM

Means within columns with different superscripts are different (P<0.05)

Table 3. Effect of maturity stage of alfalfa, medium pH and level of fibrolytic enzyme on *in vitro* digestibility of hemicellulose and cellulose.

		Enzyme [1]					
		0		1		2	
Maturity stage	Medium pH	Hemicellulose	Cellulose	Hemicellulose	Cellulose	Hemicellulose	Cellulose
Early bud	6.74	74.4	59.3	74.8	54.2	75.5	51.1
	6.15	74.9	58.1	72.4	58.1	72.8	54.2
	5.39	71.3	50.1	73.6	47.3	71.1	53.8
Late bud	6.74	73.8	54.9	73.9	54.4	74.9	56.4
	6.15	71.7	47.5	72.0	53.4	75.2	58.2
	5.39	71.6	50.3	74.4	50.1	71.8	45.2
Early bloom	6.74	64.1	50.5	62.5	45.0	64.4	40.5
	6.15	63.3	43.0	60.6	45.2	63.4	44.3
	6.39	60.6	39.7	64.6	42.8	62.2	46.5

[1] 0= 0.0, 1= 0.0138 and 2= 0.0276 g of Fibrozyme®/g of forage DM
Means within columns with different superscripts are different (P<0.05)

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REST STOPS DURING ROAD TRANSPORT: IMPACTS ON PERFORMANCE AND ACUTE-PHASE PROTEIN RESPONSES OF FEEDER CATTLE

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ABSTRACT: Angus × Hereford steers (n = 42) and heifers (n = 21) were ranked by gender and BW on d 0 of the experiment, and randomly assigned to 1 of 3 treatments: 1) no transport and full access to feed and water (**CON**); 2) continuous road transport for 1,290 km (**TRANS**), or 3) road transport for 1,290 km, with rest stops every 430 km (**STOP**; total of 2 rest stops). Treatments were applied from d 0 to 1 of the study. Cattle from TRANS and STOP treatments were transported in separate commercial livestock trailers, but through the exact same route. During each rest stop, STOP cattle were unloaded and offered alfalfa-grass hay and water for ad libitum consumption for 2 h. Upon arrival of STOP and TRANS on d 1, cattle were ranked by gender and BW within each treatment and assigned to 21 pens (7 pens/treatment; 2 steers and 1 heifer per pen). Full BW was recorded prior to (d -1 and 0) treatment application and at the end of experiment (d 28 and 29) for ADG calculation. Total DMI was evaluated daily from d 1 to 28. Blood samples were collected on d 0, 1, 4, 7, 10, 14, 21, and 28. Body weight shrink from d 0 to d 1 was reduced ($P < 0.01$) in CON vs. TRANS, CON vs. STOP, and STOP vs. TRANS. Mean ADG was greater ($P < 0.05$) in CON vs. TRANS and STOP, but similar ($P = 0.68$) between TRANS and STOP. Mean G:F was greater ($P = 0.05$) in CON vs. STOP, tended to be greater ($P = 0.08$) in CON vs. TRANS, and similar ($P = 0.85$) between TRANS and STOP. Plasma cortisol was greater ($P < 0.04$) in TRANS vs. CON and STOP on d 1, and greater ($P = 0.04$) in TRANS vs. CON on d 4. Serum NEFA was greater ($P < 0.01$) in TRANS vs. CON and STOP on d 1, and greater ($P \leq 0.05$) in TRANS vs. CON on d 4 and 7. Plasma haptoglobin was greater ($P < 0.04$) in TRANS vs. CON and STOP on d 1, and STOP vs. CON on d 1. In conclusion, inclusion of rest stops during a 1,290-km transport prevented the increase in circulating cortisol and alleviated the NEFA and haptoglobin response elicited by transport, but did not improve feedlot receiving performance of transported cattle.

Key Words: Acute-phase proteins, beef cattle, feedlot receiving, rest stops

Introduction

Cattle are exposed to several psychologic, physiologic, and physical stressors associated with management procedures currently practiced within beef production systems (Carroll and Forsberg, 2007). Transporting, for example, is one of the most stressful events experienced by feeder cattle (Swanson and Morrow-Tesch, 2001). Upon long transportation periods, cattle experience inflammatory and acute-phase responses (Cooke et al., 2011) that may impair health and productivity during feedlot receiving (Araujo et al., 2010). Moreover, recent research from our group demonstrated that feed and water deprivation are major contributors to the acute-phase response and reduced feedlot receiving performance detected in transported cattle (Marques et al., 2012). Therefore, alternatives to prevent, or at least alleviate, prolonged periods of feed and water restriction during transport may modulate the acute-phase and performance responses during feedlot receiving.

A potential alternative would be adoption of rest stops during long transport for water and feed consumption, which are mandatory during 24-h or longer transports in Canada and European countries (Tarrant and Grandin, 2000; Council Regulation Nº 1/2005, 2005). However, to our knowledge, no research studies have compared the acute-phase response between cattle transported for long distances and allowed, or not, to stop for water and feed intake. In addition, rest stops may further expose cattle to stressors, including handling for loading and unloading, known to stimulate the bovine acute-phase response (Carroll and Forsberg, 2007). Hence, the objective of this experiment was to evaluate the effects of rest stops during road transport on circulating concentrations of cortisol, NEFA, acute-phase proteins, and feedlot receiving performance of feeder cattle.

Materials and Methods

The experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns Station) from October to November 2012. All animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee.

Animals and diets. Sixty-three Angus × Hereford steers (n = 42) and heifers (n = 21), which were weaned 45 d prior to the beginning of the experiment (d 0), were maintained in a single meadow foxtail (*Alopecurus pratensis* L.) pasture from d -15 to 0 for preconditioning. During this period, cattle were fed mixed alfalfa-grass hay ad libitum and 2.3 kg/animal daily (DM basis) of a concentrate containing (as-fed basis) 84% cracked corn, 14% soybean meal, and 2% mineral mix. On d 0, cattle were ranked by gender and initial BW (229 ± 2 kg; initial age = 211 ± 3 d) and assigned to 1 of 3 treatments: 1) no transport and full access to feed and water (**CON**); 2) continuous road transport for 1,290 km (**TRANS**), or 3) road transport for

1,290 km, with rest stops every 430 km (**STOP**; total of 2 rest stops). Cattle assigned to TRANS and STOP traveled from d 0 to d 1 in separate commercial livestock trailers, within a single 2.1 x 7.2 m compartment, but through the exact same route. However, STOP cattle were loaded and initiated transport 4 h before TRANS cohorts (0600 and 1000 h on d 0, respectively) so both treatment groups were unloaded at the same time on d 1 (1000 h). During each rest stop, STOP cattle had ad libitum access to mixed alfalfa-grass hay and water for 2 h before being re-loaded into the same commercial livestock trailer. Moreover, during the first rest stop (1300 to 1500 h), STOP cattle were individually allocated to drylot pens (8 × 20 m) for feed and water intake evaluation. During the second rest stop (2300 to 0100 h), STOP cattle were not individually penned due to the lack of visibility for proper animal handling, and were maintained in a single drylot pen (20 × 35 m). The CON treatment was included as a non-transport positive control for physiological and performance measurements, and remained in the same meadow foxtail pasture with ad libitum access to mixed alfalfa-grass hay and 2.3 kg/animal (DM basis) of the aforementioned concentrate while TRANS and STOP cattle were being transported.

Immediately upon arrival of STOP and TRANS groups on d 1, cattle were ranked by gender and BW within each treatment and assigned to 21 feedlot pens (7 pens/treatment; 2 steers and 1 heifer/pen; 8 × 20 m) for a 28-d feedlot receiving. During feedlot receiving, all pens were fed mixed alfalfa-grass hay ad libitum and 2.3 kg/animal daily (DM basis) of the aforementioned corn-based concentrate, which was offered separately from hay at 0800 h. Water was offered for ad libitum consumption from d -15 to 28, except to STOP and TRANS cattle when inside the livestock trailers.

All cattle were vaccinated against clostridial diseases (Clostrishield 7; Novartis Animal Health; Bucyrus, KS) and bovine virus diarrhea complex (Virashield 6 + Somnus; Novartis Animal Health) at approximately 30 d of age. At weaning (d -45), cattle were vaccinated against clostridial diseases and *Mannheimia haemolytica* (One Shot Ultra 7; Pfizer Animal Health; New York, NY), infectious bovine rhinotracheitis, bovine viral diarrhea complex, and pneumonia (Bovi-Shield Gold 5 and TSV-2; Pfizer Animal Health), and administered an anthelmintic (Dectomax; Pfizer Animal Health). No incidences of mortality or morbidity were observed during the entire experiment.

Sampling. Individual full BW was recorded and averaged over 2 consecutive days prior to treatment application (d -1 and 0) and at the end of experiment (d 28 and 29) for ADG calculation. Individual BW was also collected on d 1, immediately after treatment application, to evaluate BW shrink as percentage change from the average BW recorded on d -1 and 0. Concentrate, hay, and total DMI were evaluated daily from d 1 to 28 from each pen by collecting and weighing orts daily. Samples of the offered and non-consumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of animals within each pen, and expressed as kg per animal/d. Total BW gain and DMI of each pen from d 1 to 28 were used for feedlot receiving G:F calculation.

Blood analysis. Blood samples were collected on d 0 (prior to loading of TRANS and STOP cattle), 1 (immediately after unloading of TRANS and STOP cattle), and on d 4, 7, 10, 14, 21, and 28, via jugular venipuncture into commercial blood collection tubes with or without 158 USP units of freeze-dried sodium heparin for plasma and serum collection, respectively. Blood samples were collected prior to concentrate feeding, except for d 0 when STOP and TRANS cattle were transported immediately after blood collection. All blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection. Plasma concentrations of cortisol were determined in samples collected from d 0 to d 10 using a bovine-specific commercial ELISA kit (Endocrine Technologies Inc., Newark, CA). Plasma concentrations of ceruloplasmin and haptoglobin were determined in all samples according to colorimetric procedures previously described (Demetriou et al., 1974; Cooke and Arthington, 2012). Serum concentrations of NEFA were determined in samples collected from d 0 to d 10 using a colorimetric commercial kit (HR Series NEFA – 2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA). The intra- and inter-assay CV were, respectively, 8.0 and 7.2% for cortisol, 3.7 and 5.8% for NEFA, 9.4 and 6.2% for ceruloplasmin, and 7.2 and 6.5% for haptoglobin.

Statistical analysis. Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for BW shrink and ADG contained the effects of treatment, gender, and the treatment × gender interaction. Data were analyzed using animal(treatment × pen) as random variable. The model statement used for DMI and G:F contained the effects of treatment, as well as day and the treatment × day interaction for DMI only. Data were analyzed using pen(treatment) as the random variable. The model statement used for blood variables contained the effects of treatment, day, gender, all resultant interactions (treatment × gender, treatment × day, and treatment × gender × day), and values obtained on d 0 as covariate. Data were analyzed using animal(treatment × pen) as the random variable. The specified term for the repeated statements was day, pen(treatment) or animal(treatment × pen) as subject for DMI or blood variables, respectively, and the covariance structure utilized was based on the Akaike information criterion. Results are reported as least square means, as well as covariately adjusted least square means for blood variables, and were separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main effects if no interactions were significant, or according to the highest-order interaction detected.

Results and Discussion

No interactions containing the effects of treatment and gender were detected ($P \geq 0.44$) for the variables

analyzed and reported herein; therefore, results are reported across steers and heifers. A treatment effect was detected ($P < 0.01$) for BW shrink from d 0 to 1. Shrink was greater ($P < 0.01$) for both TRANS and STOP compared with CON cattle, but also greater ($P < 0.01$) for TRANS compared with STOP cattle (Table 1). Previous research from our group reported equivalent BW shrink rates in feeder cattle exposed to the same transportation schedule as TRANS cattle were exposed to herein (Marques et al., 2012). Supporting our hypothesis, the rest stop schedule effectively allowed STOP cattle to consume water and feed, and consequently reduced the BW shrink resultant from the 1,290-km transport. During the first rest stop, all STOP cattle immediately consumed water whereas hay DMI was 1.49 ± 0.3 kg per animal. During the second rest stop, water consumption of STOP cattle was not monitored due to the lack of visibility, but average hay DMI was 1.64 kg per animal. However, the STOP treatment did not benefit feedlot receiving performance based on the treatment effects detected ($P = 0.05$) for ADG and G:F ($P = 0.10$; Table 1). Cattle assigned to CON had greater ADG compared with TRANS ($P = 0.05$) and STOP ($P = 0.02$) cohorts, whereas ADG was similar between ($P = 0.68$) TRANS and STOP cattle, corroborating that feedlot receiving ADG is reduced in transported cattle compared to non-transported cohorts (Marques et al., 2012). Still, treatment effects detected on ADG were not sufficient to impact ($P = 0.56$) cattle BW at the end of the experimental period (Table 1). No treatment effects were detected ($P \geq 0.18$) on hay, concentrate, and total DMI (Table 1), although other authors reported depressed feed intake in cattle upon road transport (Hutcheson and Cole, 1986) due to impaired ruminal function and altered endocrine or metabolic patterns (Cole, 2000). Nevertheless, CON had greater G:F compared with STOP ($P = 0.05$) and tended to have greater G:F compared with TRANS cattle ($P = 0.08$), whereas G:F was similar ($P = 0.85$) between TRANS and STOP cattle (Table 1). Hence, STOP cattle experienced a similar decrease in feedlot receiving performance compared with TRANS cohorts, indicating that 2-h rest stops failed to alleviate the performance losses caused by road transport.

Table 1. Feedlot receiving performance (28 d) of cattle assigned to continuous road transport for 1,290 km (**TRANS**), road transport for 1,290 km but with rest stops every 430 km (**STOP**), or no transport and full access to feed and water (**CON**)[1].

Item	CON	FM	TRANS	SEM	P =
BW, kg					
Initial	230	229	229	4	0.96
Final	268	261	262	5	0.56
Shrink, %	-1.25[a]	5.82[b]	10.17[c]	0.47	< 0.01
ADG, kg/d	1.28[a]	1.09[b]	1.13[b]	0.06	0.05
DMI, kg/d					
Hay	5.17	4.95	5.17	0.10	0.21
Concentrate	2.30	2.27	2.30	0.01	0.26
Total	7.48	7.21	7.47	0.11	0.18
G:F, g/kg	533[a]	465[b]	471[ab]	24	0.10

[1] Within rows, values with different superscripts differ ($P < 0.05$).

Plasma cortisol concentrations were greater ($P < 0.04$) in TRANS vs. CON and STOP on d 1, and greater ($P = 0.04$) in TRANS vs. CON on d 4 (Figure 1; treatment × day interaction, $P = 0.09$), indicating that the STOP treatment prevented the transport-elicited increase in plasma cortisol during feedlot receiving (Cooke et al., 2011).

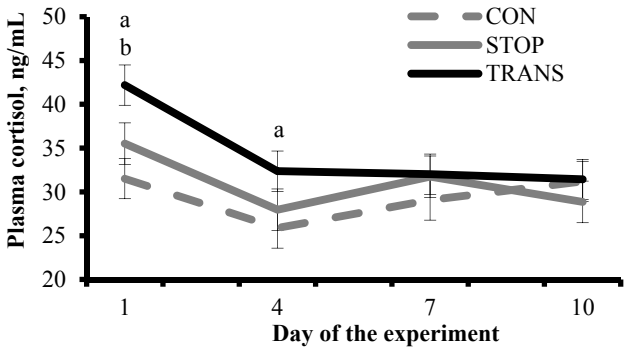

Figure 1. Plasma cortisol concentrations in cattle assigned to continuous road transport for 1,290 km (**TRANS**), road transport for 1,290 km but with 2-h rest stops every 430 km (**STOP**), or no transport and full access to feed and water (**CON**). A tendency for a treatment × day interaction was detected ($P = 0.09$). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ($P \leq 0.04$), b = TRANS vs. STOP ($P = 0.04$).

Serum NEFA concentrations were greater ($P < 0.01$) in TRANS vs. CON and STOP on d 1, and greater ($P \leq 0.05$) in TRANS vs. CON cattle on d 4 and 7 (Figure 2; treatment × day interaction, $P < 0.01$). Serum NEFA concentrations directly reflect the amount of fat tissue mobilization caused by feed restriction during road transport (Earley and O'Riordan, 2006; Marques et al., 2012). Hence, the STOP treatment effectively reduced, but did not eliminate, fat tissue mobilization by allowing cattle to consume feed during the 2-h rest stops and preventing the transported-induced increase in serum cortisol.

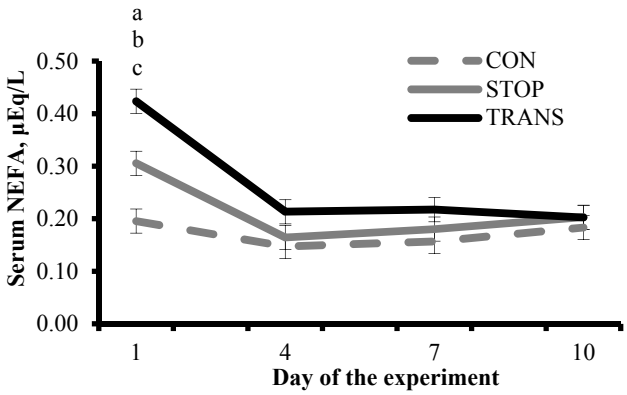

Figure 2. Serum NEFA concentrations in cattle assigned to continuous road transport for 1,290 km (**TRANS**), road transport for 1,290 km but with 2-h rest stops every 430 km (**STOP**), or no transport and full access to feed and water (**CON**). A treatment × day interaction was detected ($P < 0.01$). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ($P \leq 0.05$), b = TRANS vs. STOP ($P < 0.04$), c = STOP vs. CON ($P < 0.01$).

During feedlot receiving, mean plasma ceruloplasmin concentrations were similar (P = 0.19) between treatments (35.4, 37.8 and 36.2 mg/dL for CON, STOP, and TRANS, respectively; SEM = 0.95). Plasma haptoglobin concentrations were greater (P < 0.04) in TRANS vs. CON and STOP cattle on d 1, as well as greater (P = 0.04) in STOP vs. CON cattle on d 1 (Figure 3; treatment × day interaction, P = 0.08). Previous research from our group also documented an increase in plasma haptoglobin concentrations in beef cattle upon a similar road transport (Araujo et al., 2010; Francisco et al., 2012) that impaired feedlot receiving ADG and G:F (Marques et al., 2012). Accordingly, circulating concentrations of haptoglobin in transported feeder cattle have been negatively associated with feedlot receiving performance (Araujo et al., 2010). However, the same treatment response was not detected for plasma ceruloplasmin concentrations, although plasma concentrations of ceruloplasmin and haptoglobin are typically correlated (Cooke et al., 2009).

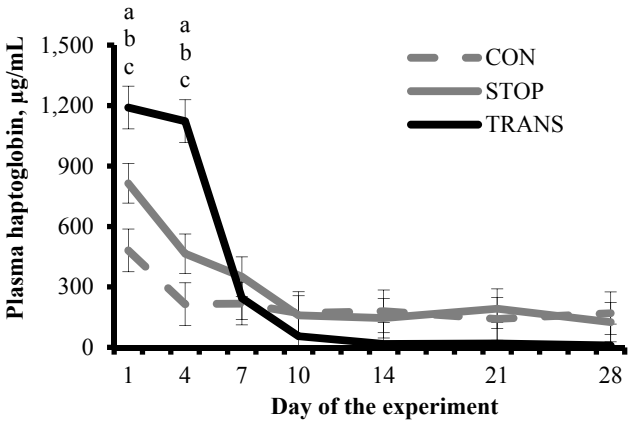

Figure 3. Plasma haptoglobin concentrations in cattle assigned to continuous road transport for 1,290 km (**TRANS**), road transport for 1,290 km but with 2-h rest stops every 430 km (**STOP**), or no transport and full access to feed and water (**CON**). A treatment × day interaction was detected (P < 0.01). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ($P \leq 0.05$), b = TRANS vs. STOP (P < 0.01), c = STOP vs. CON (P < 0.01).

Implications

In conclusion, providing 2-h rest stops during a 1,290-km transport prevented the increase in circulating cortisol and alleviated the NEFA and haptoglobin response elicited by transport, but did not improve feedlot receiving performance of transported cattle. Hence, rest stops appear to be an alternative to reduce neuroendocrine and acute-phase protein responses during transport and feedlot receiving.

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ANALYSIS OF SIX NEUROPEPTIDES DETECTED IN HYPOTHALAMUS AND PITUITARY GLAND OF PRE- AND POST-PUBERTAL BRANGUS HEIFERS

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ABSTRACT: Discovery of genes associated with onset of puberty could facilitate earlier identification of replacement females in beef production systems. We evaluated expression of six neuropeptides (PCSK1N [221-240], PCSK1N[61-89], CBLN1[58-71], CART[28-36], PDNY[186-208], and CMGA[334-347]) in hypothalamus and pituitary glands of pre- (PRE) and post- (POST) pubertal Brangus heifers. Pubertal stage was determined via circulating progesterone concentration (PRE <1 ng/mL; POST = 2 consecutive progesterone values > 1 ng/mL). Slaughter age and BW were 353 ± 23.85 and 437 ± 16.51 days of age and 272.59 ± 8.78 and 354.66 ± 9.51 kg for PRE and POST heifers, respectively. Hypothalamus and pituitary glands were harvested and the peptidome (population of neuropeptides ≤ 10 kDa) of each organ were explored. Subsequently, quantification was performed using multiple-reaction monitoring mass spectrometry of peptide extracts. Expression of PCSK1N [221-240], PCSK1N[61-89] and CART[28-36] were greater ($P < 0.05$) in POST pituitary glands relative to PRE heifers (i.e., peak area estimates were 1,118,058 ± 91,847 > 65,504 ± 8,761 for PCSK1N [221-240]; 2,308,678 ± 71,117 > 490,032 ± 11,969 for PCSK1N[61-89]; 7,137,698.33 ± 573,490.28 > 534,219.33 ± 228,144.76 for CART[28-36]). Hypothalamus was greater ($P < 0.05$) than pituitary gland expression in POST heifers for CBLN1[58-71] (i.e., 137,304 > 33,572 ± 20,154.78) and in PRE heifers for PDNY[186-208] (i.e., 231,291.667 > 6,070.667 ± 14,437.60). Within pituitary glands, POST females expressed 73.62% ($P = 0.0482$) more CMGA[334-347]) peptides than PRE heifers. Peptides PCSK1N [221-240] and PCSK1N[61-89] are products of the *PCSK1N* gene, which have biological gene ontology specific to peptide hormone processing. The CART [28-36] and PDNY [186-208] peptides have function in neuropeptide signaling pathways; thus, should be considered a positional and functional candidate for future reproduction studies.

Key words: bovine, heifer, puberty, neuropeptidome

Introduction

Puberty in beef heifers is dependent on the interaction of endocrine events, environmental influences, genetics, and nutritional status. Induction of the pre-ovulatory GnRH surge, triggered by estradiol, is an endocrine axis essential for puberty (Kinder et al., 1987). Puberty timing is not absolute and occurs at older ages in *Bos indicus* and *Bos indicus*-influenced females compared to *Bos taurus* cattle (Johnston et al., 2009). Identification of neuropeptide expression differences in beef heifers prior to and after pubertal onset will contribute to our understanding of this biological process.

Hypothalamic identification of neuropeptides was recently conducted with mass spectrometry (MS) technology (Colgrave et al., 2011), which can detect multiple neuropeptides in a single sample with high sensitivity. Previously, we conducted a qualitative analysis of neuropeptides present in hypothalamus and pituitary glands of pre- and post-pubertal Brangus heifers (DeAtley et al., 2013), which identified 240 neuropeptides. Objectives of this study utilized these results, findings of Colgrave et al. (2011), and previous literature to conduct quantitative analysis of six neuropeptides (i.e., PCSK1N [221-240], PCSK1N[61-89], CBLN1[58-71], CART[28-36], PDNY[186-208], and CMGA[334-347]) that may be important in puberty of Brangus heifers.

Materials and Methods

Animals were handled and managed according to Institutional Animal Care and Use Committee of New Mexico State University (NMSU).

Eight Brangus (3/8 Brahman x 5/8 Angus) heifers were selected from the NMSU Breeding herd (Luna-Nevarez et al., 2010) and were categorized into two physiological states; pre-pubertal (PRE; n = 4) and post-pubertal (POST; n = 4). Pubertal state determination was based on circulating progesterone concentration (i.e., PRE = less than 1 ng/mL; POST = 2 consecutive progesterone values > 1 ng/mL; Shirley et al., 2006). Pre-pubertal and POST heifers were slaughtered at 353 ± 23.85 and 437 ± 16.51 d of age, respectively. Post-pubertal heifers were synchronized with 5 mL $PGF_2\alpha$ (Lutalyse; Pfizer, New York, NY), subsequent estrus observed, and slaughtered 10 d post estrus. Procedures of Narro et al. (2003) were used in dissection of hypothalamic tissue. The pituitary gland (i.e., anterior and posterior) was also collected and both tissues were obtained within 15 min *postmortem* and snap-frozen in liquid nitrogen then stored at -80° C until processing. Frozen hypothalamus and pituitary glands were further dissected to 5mm diameter, stabilized with the Stabilizor T1 (Denator, Göteborg, Sweden) similar to procedures of Colgrave et al. (2011), and stored at -80˚C until extraction.

Hypothalamus and pituitary gland peptides were extracted similar to procedures of Colgrave et al. (2011). Briefly, hypothalamus samples were processed in four fractions and pituitary gland samples processed in two

fractions of 35 mg of tissue per sample. Individual fractions were suspended in a cold acetic acid extraction solution (11.43 µL 0.25% acetic acid/mg tissue) and homogenized using microtip sonication (60 Sonic Dismemrator, Fisher Scientific, Pittsburgh, PA). Fractions were then centrifuged at 20,000 x *g* for 30 min. Supernatant was collected and applied to a Nanosep 10kDa Omega filter (Pall Corporation, Ann Arbor, MI) then samples were centrifuged at 20,000 x *g* for 90 min. Filtrate was collected and concentrated with a SpeedVac concentrator (Thermo Scientific, Pittsburgh, PA) to 20 µL volume and stored at -4˚C. Remaining tissue from the first centrifugation process was re-suspended in 200 µL 0.25% acetic acid wash and re-centrifuged for 30 min; supernatant was collected and applied to the 10 kDa filter then centrifuged for 90 min at 20,000 x *g* (i.e., first wash). An additional 100 µL 0.25% acetic acid was added to filter as a second wash and centrifuged for 30 min. In total, ~300 µL of filtrate (~200 µL from first wash and 100 µL from second wash) were collected and concentrated to 20 µL volume and stored at -80˚C until MS analyses.

Multiple reaction monitoring (MRM) procedures were used for quantification of six peptides, which were previously identified in qualitative analyses (DeAtley et al., 2013). Peptides quantities were analyzed on an Applied Biosystems 4000 QTRAP mass spectrometer (Applied Biosystems, Framingham, MA) equipped with a nanospray II ionization source operated in positive ion mode. Samples were chromatographically separated on an Eksigent Nano 1D Plus system (Dublin, CA) using a Proteopep II column (150 mm x 75 µm) with a particle size of 5 µm and a linear gradient of 5-40% acetonitrile over 40 min with a flow rate of 300 nL/min. The eluent for the HPLC was coupled directly to the mass spectrometer. Data were acquired and processed using Analyst v1.5.2 software (AB/Sciex, Framingham, MA). Scan type was set to MRM and source conditions were: ionspray voltage 2300 V, curtain gas 20, GS1 25, GS2 0, interface temperature 150°C and resolution was set to low for both Q1 and Q3. Transitions were built using spectral information obtained in the qualitative analysis (DeAtley et al., 2013). Collision energy was set according to the mass and charge of each precursor ion. The MRM transitions were scheduled based on their expected retention time using a 4 min window with a 1.5 s cycle time. The MRM procedures were used for quantification of selected neuropeptides by integrating the peak area of the most intense MRM transition for each peptide using MultiQuant software (AB/Sciex, Framingham, MA). The average peak area was determined by taking the mean of three replicate injections (i.e., technical replicates).

Average peak area data for the six neuropeptides in each tissue (i.e., hypothalamus or pituitary gland) and pubertal treatment (i.e., PRE or POST) were analyzed with a one-way ANOVA and the PROC GLM procedures of SAS (version 9.2, SAS Inst., Inc., Cary, NC). The Uniprot database (www.uniprot.org) was used to characterize each of the six peptides (i.e., precursor and gene identification, molecular weight, amino acid sequence, and gene ontology).

Results

In this study, a total of 5 precursors were represented; PCSK1N [221-240] and PCSK1N [61-89] peptides are products of the same precursor (i.e., PSCK1N). The integrated peak area indicative of peptide expression level of each of the neuropeptides in hypothalamus and pituitary glands of PRE and POST Brangus heifers are shown in Figure 1. Expression of PCSK1N [221-240] was 70.71% ($P = 0.0058$) greater in POST pituitary glands compared to hypothalamus and 94.14% ($P < 0.01$) greater than peak area detected in PRE pituitary gland. Similarly, PSCK1N [61-89] was expressed at a higher rate in POST pituitary gland vs. hypothalamus ($P < 0.01$). Pre-pubertal heifers expressed more total PSCK1N [61-89] peptides in pituitary gland than hypothalamus ($P = 0.04$); however, peak area was significantly ($P < 0.01$) less in PRE compared to POST pituitary glands. Peptides PCSK1N [221-240] and PCSK1N [61-89] were both detected in the greatest quantity in POST pituitary glands. Cerebellin-1 [58-71] expression was greatest in POST hypothalamus vs. pituitary gland ($P = 0.0220$); conversely, PRE heifers had greater total peak area within pituitary gland than POST ($P = 0.0484$). Neuropeptide CART [28-36], the smallest peptide evaluated in this study, was detected in minute amounts in hypothalamus; however, in POST pituitary glands a greater proportion (92.51%; $P < 0.001$) of CART [28-36] peptides were detected compared to PRE heifers.

The PDNY [186-208] peptide, within the PRE state, was almost exclusively detected in hypothalamus ($P = 0.0004$). Expression levels of PRE hypothalamus were 36.58% greater than POST. Finally, CMGA[334-347], a peptide of the chromogranin-A precursor, was expressed in similar ($P > 0.05$) levels in hypothalamus tissue between PRE and POST stages, but decreased peak areas were detected in PRE pituitary gland samples ($P = 0.04$) compared to hypothalamus. Within pituitary gland tissue, POST females expressed 73.62% ($P = 0.0482$) more CHGA[334-347] peptides than PRE heifers.

Discussion

Six neuropeptides (i.e., PCSK1N [221-240], PCSK1N[61-89], CBLN1[58-71], CART[28-36], PDNY[186-208], and CMGA[334-347]) were selected for quantitative analysis based on (1) their presence in previous qualitative analyses of hypothalamus and pituitary glands of PRE and POST heifers (DeAtley et al., 2013); (2) their detection in hypothalamus of two multiparous, lactating *Bos indicus* cows (Colgrave et al., 2011); and (3) previous reports which had reported their relevance in neuroendocrine function (Lee et al., 2010; Wardman et al., 2011).

Peptides PCSK1N [221-240], and PCSK1N[61-89] are derived from 27 kDa precursor, ProSAAS (PCSK1N; Wardman et al., 2011), which is encoded by the proprotein convertase subtilisin/kexin type 1 inhibitor (*A4IFR2*) gene on bovine chromosome X. Biological gene ontology for ProSAAS-derived peptides are linked to peptide hormone processing function. Peptides are produced via cleavage of larger precursor proteins at

dibasic residues (i.e., Arg-Arg or Lys-Arg). Endopeptidases (i.e., prohormone convertases) cleave the precursors at these sites, which generates intermediate peptides with C-terminal basic amino acid extensions. Carboxypeptidases then trim these residues allowing for post-translational modifications (i.e., amidation) before becoming biologically active. Carboxypeptidase E (CPE) is found in all neuroendocrine tissues and cleaves many C-terminal-extended peptides to generate an active structure. The CPE peptides are involved in biosynthesis of proSAAS-derived peptides and processing takes place in the regulated secretory pathway of neuroendocrine cells (Wardman et al., 2011). Results of this puberty study detected PCSK1N [221-240] and PCSK1N[61-89] in both hypothalamus and pituitary glands of PRE and POST pubertal heifers. Peptide detection in multiple tissues coupled with greater proportion of expression in POST pituitary glands indicate that neuropeptide processing is taking place in both hypothalamus and pituitary gland.

Cerebellin [58-71] is a 13 amino acid peptide, whose gene, *CBLN1*, is located on bovine chromosome 18. The cerebellin [58-71] peptide is missing one amino acid residue from both the N- and C-terminal portions. If fully intact (i.e., SGSAKVAFSAIRSTNH) then the 16 aa peptide is classified as Cerebellin and thought to act as a neurmodulator. The Cerebellin-1 glycoprotein is required for synapse integrity and plasticity, which has been shown to rapidly induce synaptogenesis and is necessary for maintaining normal synapse in the cerebellum *in vivo* (Hirai et al., 2005). In this study, Cerebellin-1 [58-71] was detected in all tissue and pubertal state samples. Post-pubertal pituitary samples expressed smaller proportions of the neuropeptide than PRE pituitary glands and both pubertal stages in hypothalamus. Since no difference was detected between PRE and POST hypothalamus expression of Cerebellin-1 [58-71], it may serve as a neuron housekeeping peptide that is commonly expressed to maintain synaptic integrity.

Cocaine- and amphetamine-regulated transcript (CART) peptides are neurotransmitters that have been associated with cattle traits; specifically, feeding behavior and BW regulation (Zhang et al., 2008). The CART peptide is cleaved into two chains, CART [1-39] and CART [42-89] (http://www.uniprot.org/uniprot/Q68RJ9). The peptide analyzed in this study, CART [28-36], is the C-terminal portion of CART [1-39]. The *CART* gene, located on bovine chromosome 20, and has vast biological ontogeny including neuropeptide signaling pathway function (Roa and Herbison, 2012). In puberty studies, *CART,* has been associated with energy homeostasis, which is controlled by several nuclei in the arcuate nucleus (ARC) and is regulated by leptin, reduced feed intake, and increased catabolic processes (Gautron and Elmquist, 2011). At the pituitary level, our findings and those of Kappeler et al. (2006) both observed similar CART peptide expression; however, the animal model used by Kappeler et al. (2006) was adult male Wistar rats. Therefore, increased pituitary gland expression of CART peptides may be a result of sex or species differences.

The PDNY-[186-208] peptide is a 22 amino acid peptide whose gene is located on bovine chromosome 13 and has neuropeptide signaling and opioid peptide activity. Specifically, the gene *EIBDY2* codes for PDNY-[186-208] and has been implicated as part of the kisspeptin-neurokinin B-dynorphin (KNDy) subpopulation in the ARC. The KNDy cells are a major target for steroid hormones and form an intricate signaling network, which convey steroid feedback to GnRH neurons and potentially serve as a component of the GnRH pulse generator. Kisspeptin transmits steroid feedback signals to GnRH neurons, especially the positive feedback effect of estradiol that causes the preovulatory GnRH/LH surge (Wakabayashi et al., 2010). In the current study, PDNY-[186-208] was expressed in greater concentrations in PRE hypothalamus vs. POST; however, the opposite trend was observed in pituitary gland where POST heifers had significantly greater expression. Increased hypothalamic expression in PRE females disagree with reports of Muneoka et al. (2009), which found increased dynorphin expression prior to puberty in male rat hypothalamus. These contradicting results imply that dynorphin may be expressed in differential patterns depending upon sex. Expression of PDNY-[186-208] in POST pituitary glands were similar to a trend reported by Escobar et al. (2012) with *Kiss1* mRNA in European sea bass. Specifically, mRNA expression was detected for *Kiss1* in FSH-β positive cells, but not in LH-β positive cells. These results coupled with increased expression in POST pituitary heifers indicate that kisspeptin and dynorphin may also interact within a signaling network system in the pituitary gland.

The chromogranin-A (i.e., *CHGA*) gene codes for the peptide CMGA[334-347], a 14 amino acid peptide, and is a member of the neuroendocrine secretory protein family located in neuron secretory vesicles. In our puberty model we observed varied expression of CMGA[334-347]; specifically, in pituitary glands, POST heifers had greater concentrations than PRE. Also, hypothalamus concentrations of CMGA[334-347] were similar among pubertal status. Because this peptide was detected in this study and in the hypothalamus samples of mature *Bos indicus*-influenced cows (Colgrave et al., 2011), this may indicate that the peptide is commonly expressed across breed types and physiological status (i.e., pubertal vs. mature cow).

In summary, six neuropeptides (i.e., PCSK1N [221-240], PCSK1N[61-89], CBLN1[58-71], CART[28-36], PDNY[186-208], and CMGA[334-347]) were quantified via MS procedures. The PCSK1N [221-240] and PCSK1N[61-89] peptides were derivatives of the *A4IFR2* gene, which appear to be involved in peptide hormone processing. We also showed that CART [28-36] was minimally expressed in hypothalamus, but overexpressed in POST pituitary glands. The PDNY [186-208] peptide, a product of the prodynorphin precursor, functions as part of the kisspeptin-neurokinin B-dynorphin cell signal network a known regulator of puberty. Also, cerebellin [58-71] and CMGA[334-347] peptides may be housekeeping-type peptides due to their biological ontology and non-specificity to pubertal and reproductive traits. Cumulatively, results of this study provide preliminary data to develop new expression studies of these peptides in other cattle breeds and experimental models.

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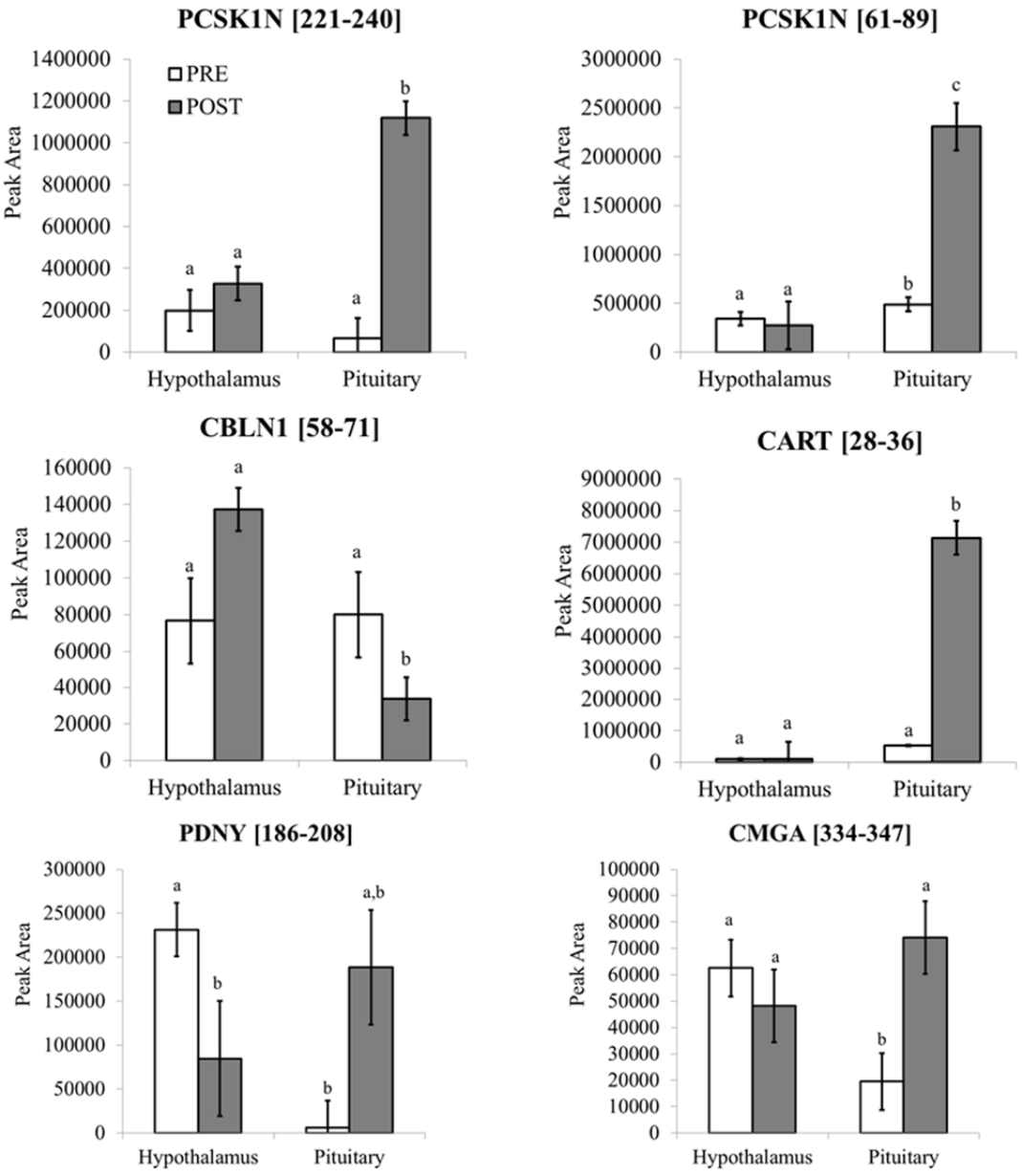

Figure 1. Peak area for PCSK1N [221-240], PCSK1N [61-89], Cerebellin-1 [58-71], CART [28-36], PDNY [186-208], and CMGA [334-347] neuropeptides detected in hypothalamus and pituitary glands of pre- (n = 3) and post- (n = 3) pubertal Brangus (3/8 Brahman x 5/8 Angus) heifers. Superscripts (a,b) differ $P < 0.05$.

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EFFECT OF FLUNIXIN MEGLUMINE ON FIRST SERVICE CONCEPTION RATE IN BEEF HEIFERS

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ABSTRACT: The objective of this study was to determine if post-breeding injection of flunixin meglumine would improve first service conception rate in beef replacement heifers. Following weaning, heifers (n = 220; BW = 367 ± 37 kg; BCS = 5.3 ± 0.04) originating from the Commercial Cow-Calf Unit (Manhattan, KS) and Agricultural Research Center (Hays, KS) were developed in the feedlot at Hays, KS and then returned to their respective origins for the breeding season. Heifers were stratified by origin, BW, and BCS and assigned randomly to one of two treatments: 1) injection with physiological saline (**CON**); or 2) injection with flunixin meglumine (**BAN**). Injections were administered subcutaneously at 1.1 mg/kg BW 14 d post fixed-time AI (**FTAI**). Serum progesterone (**P4**) concentrations were determined from paired blood samples collected 10 d prior and on the day ovulation synchronization was initiated to determine proportion of pubertal heifers. Ovulation was synchronized using a 7 d CO-Synch + controlled internal drug release (**CIDR**) protocol. Heifers were exposed to FTAI 60-64 h after CIDR removal and exposed to fertile bulls 10 d after FTAI for the remaining 35 d of a 45-d breeding season. Transrectal ultrasonography was used to determine conception to FTAI 35 d after insemination and final pregnancy rate was determined 35 d after the breeding season. Heifer BW and BCS at breeding were not different ($P > 0.40$) between treatments. Proportion of pubertal heifers (98.2%) did not differ between treatments ($P = 0.98$). First service conception rate of CON heifers (42.3%) did not differ ($P = 0.87$) from that of BAN heifers (41.2%). Final pregnancy rate was similar ($P = 0.39$) for CON and BAN heifers and averaged 81.8%. Under the conditions of our study, flunixin meglumine injection 14 d post-FTAI did not improve first service conception or final pregnancy rate in beef replacement heifers.

KEY WORDS: Beef heifers, conception rate, flunixin meglumine

Introduction

The development of replacement heifers requires many resources (feed, labor, etc.). Therefore, the inherent cost to establish new genetics is often high. Timely conception and maintenance of the pregnancy are vital to the long-term success and retention of replacement females in the herd. Improvement of first service conception rate remains challenging for many operations, despite enhanced knowledge and use of heifer development strategies.

Facility limitations often dictate that cattle be processed and bred in a central location, and then transported to other locations for the remainder of the breeding season. The physiological stress associated with handling and transportation increases serum cortisol concentrations, which may be used as indication of animal stress (Crookshank et al., 1979). Physiological stress has been correlated with an inability for the embryo to suppress $PGF_{2\alpha}$ secretions and failure of maternal recognition (Merrill et al., 2007; Geary et al., 2010). Early embryonic loss accounts for the greatest proportion of reproductive failure. Injection of flunixin meglumine (Banamine®) at breeding or post-breeding has been demonstrated to inhibit production of $PGF_{2\alpha}$ (Guilbault et al., 1990; Merrill et al., 2007), mitigating the effect of transportation stress, and allowing for pregnancy maintenance (Merrill et al., 2004; Purcell et al., 2005; Merrill et al., 2007).

Cattle that do not require transportation after breeding are still exposed to human interaction and handling which may result in increased stress. This unavoidable stress may potentially contribute to lower first service conception rates. Therefore, the objective of this study was to evaluate the effects of flunixin meglumine on first service conception rate in non-transported replacement heifers.

Materials and Methods

All procedures involving the handling and care of animals used in this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee (protocol no. 3175).

Animals and Experimental Design. Angus-cross heifers (n = 220; BW = 367 ± 36.7 kg; BCS = 5.3 ± 0.04). Heifers originating from the Kansas State University commercial cow-calf herds in Manhattan and Hays, KS were developed at the feedlot at the

Agricultural Research Center–Hays following weaning and were returned to their respective origins for the breeding season. Prior to initiation of ovulation synchronization, heifers were stratified by origin, body weight and body condition score, and assigned randomly to one of two treatments: 1) injection with physiological saline (**CON**) or 2) injection with flunixin meglumine (**BAN**). Treatments were administered, subcutaneously at 1.1 mg/kg of body weight, 14 d after fixed-time AI (**FTAI**).

Data Collection. Heifers were weighed and BCS was assessed by two independent, qualified observers using a 9-point scale (1=extremely emaciated, 9=extremely obese; Wagner et al., 1988) on the day of ovulation synchronization and day of breeding.

Puberty Determination. Blood samples were collected from all heifers via jugular venipuncture 10 d before and on the day ovulation synchronization was initiated. Samples were collected in to 10 ml serum vacutainer tubes (BD Vacutainer™, Becton, Dickinson, and Company, Flanklin Lakes, NJ), immediately placed on ice, allowed to coagulate for 24 h at 4°C and then centrifuged (1,500 × g) for 10 min. Serum was decanted into 12 × 75 mm plastic tubes and immediately frozen (-20°C). Concentration of progesterone (**P4**) in serum was subsequently quantified using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA; Stevenson, 2011). Intra-assay and inter-assay CV were 7.0 and 8.6%, respectively and assay sensitivity was 0.009 ng/ml. Blood collected on the two sampling dates was used to verify the functional presence of a corpus luteum (when concentrations of P4 exceeded 1 ng/mL) at the onset of ovulation synchronization. If any 1 of the 2 samples contained P4 >1 ng/mL (typical of heifers that have attained puberty and in the luteal phase of the estrous cycle), heifers were assumed to be pubertal before the onset of ovulation synchronization treatment (d 7). If concentrations in the 2 samples were <1 ng/mL heifers were considered to be pre-pubertal.

Ovulation Synchronization and Breeding. Ovulation was synchronized using the 7 d Co-Synch + controlled internal drug release (**CIDR**; EAZI-Breed CIDR®, Pfizer Animal Health, New York, NY) protocol (Larson et al., 2006). Heifers received 100 µg of GnRH intramuscularly (d -10; 2 mL Cystorelin; Merial Duluth, GA) and a CIDR (EAZI-Breed CIDR containing 1.38 g of P4) insert followed in 7 d by an injection of 25 mg prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) intramuscularly (d -3; 5 mL of Lutalyse; Pfizer Animal Health) and the CIDR was removed followed in 54 h by FTAI and a second 100 µg injection of GnRH (d 0). Heifers were exposed to fertile bulls 10 d after FTAI for the remainder of the 45-d breeding season.

First service conception rate (**FSCR**) was determined 35 d after FTAI. Pregnancy was confirmed by transrectal ultrasonography (Aloka 500V, 5MHz transrectal transducer, Wallingford, CT). A positive pregnancy outcome required the presence of uterine fluid and an embryo with a heartbeat. A final pregnancy diagnosis (**PR**) was determined 35 d after the end of the breeding season via transrectal ultrasonography.

Statistics. Body weight and BCS were analyzed using the GLM procedure of SAS (SAS Inc., Cary, NC). Reproductive data were analyzed using the CATMOD procedure of SAS (SAS Inc., Cary, NC). Differences were considered significant at $P \leq 0.05$.

Results and Discussion

Heifer BW and BCS at breeding were not different among treatments (P = 0.41 and 0.60, respectively; Table 1). Proportion of heifers pubertal before onset of ovulation synchronization was 98.2% and was not different (P = 0.99) between treatments. Treatment with BAN did not affect (P > 0.35) FSCR or PR (Table 1).

Blocking $PGF_{2\alpha}$ secretions by interferon-tau 12-17 d post-breeding allows for maternal recognition of pregnancy (Bazer et al., 1991; Roberts et al., 1992; Thatcher et al., 1994). The proposed action of flunixin meglumine administration following FTAI has been reported to promote maternal recognition of pregnancy by inhibiting cyclooxygenase, thus preventing conversion of arachadonic acid to $PGF_{2\alpha}$ (Anderson et al., 1990; Odensvik, 1995). This could potentially decrease early embryonic loss. Our data agrees with Rabaglino et al. (2010) who found no difference in FSCR among flunixin meglumine-treated versus non-treated dairy heifers (60.8% and 59.4%, respectively).

Conversely, Merrill et al. (2007) found that flunixin meglumine treatment increased AI pregnancy rate (71%) of beef cows compared to untreated females (61%). However, these beef cows were subjected to transportation stress, unlike the current study. It is possible that the elevated stress associated with transportation can be mitigated by flunixin meglumine; whereas elevated stress associated with standard breeding procedures and handling may be less severe, thus negating the potential positive effects of flunixin meglumine. Geary and coworkers (2010) concluded that handling stress in heifers may have a greater effect on embryonic inhibition of PGF_{2a} compared to cows. These authors also hypothesized that a single injection of flunixin meglumine may be inadequate to overcome the effects of stress in heifers.

Under the conditions of our study, injection of flunixin meglumine did not improve FSCR or PR of non-transported beef heifers.

Implications

Previous research demonstrated improved first service conception rate following flunixin meglumine administration in conjunction with specific conditions (i.e. where transportation of recently bred females is necessary). However, when transportation is not necessary, the use of flunixin meglumine did not improve first service conception rate of beef heifers. The cost of flunixin meglumine injection only adds to total reproductive costs, and without an accompanying improved pregnancy rate, that cost is unnecessary.

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Table 1. Reproductive performance of heifers injected with flunixin meglumine (BAN) or physiological saline (CON) 14-d post-breeding.

	Treatment			
Item	BAN	CON	SEM	*P*-value
Number of heifers	109	111		
BW, kg	365.0	369.1	2.49	0.41
BCS	5.3	5.3	0.04	0.60
FSCR[1], %	41.2	42.3	0.05	0.87
PR[2], %	84.4	79.3	0.04	0.39

[1] First-service conception rate
[2] Final pregnancy rate

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EFFECT OF FEED EFFICIENCY CLASSIFICATION ON mRNA EXPRESSION OF ANGIOGENIC FACTORS IN THE JEJUNUM OF FINISHING STEERS

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ABSTRACT: We hypothesize that a portion of the individual differences observed for feed efficiency can be attributed to small intestinal growth and function. The objective of this study was to investigate jejunal mRNA expression of angiogenic factors in high and low efficiency steers. Hereford x Angus steers (n = 59) from a single contemporary group (birth through finishing) were fed a finishing diet (11.4% CP, 2.0 Mcal NE_m/kg, 1.35 Mcal NE_g/kg; DM basis) for 57 d using the GrowSafe Feed Intake System. Residual feed intake (RFI) was calculated as the difference between actual feed intake and expected feed intake of each individual, where expected intake was determined by regressing ADG and metabolic midweight on actual feed intake. At the end of the feeding period, the 20% most efficient (low RFI, n = 8) and 20% least efficient (high RFI, n = 8) steers with 12th rib fat thickness ≥ 1.02 cm were selected for slaughter. Visceral organs were dissected, and jejunal mucosa was flash-frozen. Real-time RT-PCR was performed to determine jejunal mRNA expression of vascular endothelial growth factor (*VEGF*), *VEGF* receptors (*FLT* and *KDR*), endothelial nitric oxide synthase 3 (*NOS3*), and soluble guanylate cyclase (*GUCY1B3*). Data were analyzed with PROC MIXED in SAS 9.2 using RFI class (high vs. low efficiency) as a fixed effect. Additionally, PROC CORR was used to determine the relationship of G:F and intake with angiogenic factor mRNA expression. It was previously reported that high efficiency steers had less relative small intestinal mass than low efficiency steers (7.63 vs. 8.50 ± 0.28 g/kg BW; P = 0.04). Jejunal mRNA expression of *VEGF* (P = 0.14), *FLT* (P = 0.58), *KDR* (P = 0.40), *NOS3* (P = 0.45), and *GUCY1B3* (P = 0.47) was unaffected by RFI class, however. Intake ($P \geq 0.16$) and G:F ($P \geq 0.19$) were not correlated with angiogenic factor expression. In this study, mRNA expression of the selected angiogenic factors was unaffected by RFI class and was not associated with G:F or intake. Although small intestinal size may explain some variation in metabolic efficiency, expression of *VEGF* and *NOS* systems do not appear to substantially contribute to these differences.

Key words: angiogenic factors, residual feed intake, small intestine

Introduction

Intake and nutrient assimilation are crucial to whole animal feed efficiency. The small intestine is the main site of post-ruminal nutrient absorption. In beef cattle, it has been previously reported that a decreased jejunal mass and increased mucosal density of the jejunum are associated with improved efficiency (Meyer et al., 2012b). Blood flow is crucial to nutrient transport from the small intestine to other tissues, thus vascularity of the small intestine may affect efficiency of nutrient absorption and impact feed efficiency in the whole animal. In addition, vascularity of the small intestine has been shown to increase with increased feed intake in finishing steers (Cunningham et al., 2013).

Angiogenesis is the process of new blood vessel formation from pre-existing blood vessels. Vascular endothelial growth factor (**VEGF**) is a growth factor that regulates angiogenesis and stimulates endothelial cell survival, proliferation, and migration in conjunction with its receptors (**KDR** and **FLT**; Klagsbrun and D'Amore, 1996). Nitric oxide (**NO**) induces vasodilation, which results in increased blood flow. Endothelial nitric oxide synthase 3 (**NOS3**) produces NO, and soluble guanylate cyclase (**GUCY1B3**) is a receptor for NO that contributes to its actions (Martin et al., 2001). Not only does NO act as a vasodilator, but it also stimulates VEGF production, acting to promote angiogenesis (Roy et al., 2006). In previous studies, small intestinal mRNA expression of these angiogenic factors has been affected by diet and intake in ruminants (Neville et al., 2010; Meyer et al., 2012a,c).

Feed efficiency is influenced by multiple factors, many of which are unknown. Because the small intestine is the main site of nutrient absorption, we hypothesize that factors affecting nutrient absorption in the small intestine, such as blood flow, may contribute to feed efficiency in beef cattle. Our objective was to investigate the mRNA expression of specific angiogenic factors in the jejunum of high and low efficiency steers, as determined by RFI.

Materials and Methods

All animal procedures were approved by the University of Wyoming (**UW**) Institutional Animal Care and Use Committee.

Steer Management and Finishing Diet. Hereford x Angus steers (n = 59, 461.9 ± 4.5 kg initial BW) from one contemporary group (birth through finishing) born to the UW beef cow herd were utilized in this study. Steers were fed a finishing diet (11.4% CP, 2.0 Mcal NE_m/kg, 1.35 Mcal NE_g/kg; DM basis) using the GrowSafe System (model 4000E, GrowSafe Systems Ltd., Airdrie, AB, Canada) to record individual intake for 57 d at the UW Sustainable Agricultural Research Center in Lingle, WY. Residual feed intake (RFI) was calculated as the difference between actual feed intake and expected feed intake. Expected feed intake was determined by regressing ADG and metabolic midweight on actual feed intake (Cammack et al., 2005). At the end of the 57-d feed intake test, the

20% most efficient (low RFI, n = 8) and 20% least efficient (high RFI, n = 8) steers with a 12th rib fat thickness measuring ≥ 1.02 cm (n = 40; 558.8 ± 7.2 kg final BW) were selected for slaughter at the UW Meat Laboratory in Laramie, WY.

Tissue Collection and Visceral Organ Measurements. Selected steers (n = 16; 553.3 ± 11.8 kg final BW) were randomly allocated by efficiency group to 1 of 2 slaughter dates occurring 5 and 7 d after the end of the feed intake test so that 4 steers from both high and low efficiency groups were slaughtered on each day. Feed and water were not withheld from steers, and steers were transported (204 km) on the morning of slaughter. Steers were slaughtered using standard commercial methods, and visceral organs were removed for dissection and sampling following inspection.

The small intestine was dissected using methods of Soto-Navarro et al. (2004), stripped of digesta and fat, and weighed. During dissection a 15-cm jejunal sample was removed starting at a point adjacent to 15 cm caudal from the junction of the mesenteric and gastrosplenic vein on the mesenteric vein. The jejunal sample was cut open along the mesenteric side to expose the intestinal lumen and rinsed with warm PBS to remove digesta. Mucosal tissue was scraped from the jejunum using a glass slide, wrapped in aluminum foil, and flash-frozen in dry ice. Frozen tissues were stored at -80°C for angiogenic factor mRNA expression.

Angiogenic Factor mRNA Expression. Jejunal mucosal mRNA expression of *VEGF*, *VEGF* receptors (*FLT* and *KDR*), *NOS3*, and *GUCY1B3* was determined using quantitative real-time RT-PCR (Austin et al., 2011). Primer sequences used are given in Table 1. Frozen tissue was placed in 1 mL of TRI reagent (Sigma Chemical Co., St. Louis, MO) and homogenized using an electronic tissue grinder (IKA Laboratories; Wilmington, NC). The RNA pellet was resuspended in 100 µL RNase free water and further purified using the RNeasy kit (Qiagen, Santa Clarita, CA). The purified RNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Denver, CO).

Two micrograms of RNA (in 15 µL nuclease-free water) were mixed with 4 µL reverse transcription buffer (5X) and 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA). The mixture was placed in a thermocycler for 5 min at 25ºC, 30 min at 42ºC, 5 min at 85ºC, and held at 4ºC. The cDNA was diluted with 100 µL nuclease-free water and stored at -20ºC until semi-quantitative real-time PCR was performed. Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) such that amplicons were approximately 150 bp in size. Real-time PCR was performed by mixing 10 µL of diluted cDNA with 12.5 µL of SYBR green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), 500 pmol each of forward and reverse primer, and 0.5 µL of nuclease free water in each well of a 96-well plate. Amplification was performed using the iQ5 and 40 cycles of 95ºC for 30 sec and 60ºC for 30 sec. Melting curve analysis was performed post-amplification to ensure the quality of PCR products as noted by the presence of a single peak. Briefly, the PCR plate was heated to 95°C for 3 min and cooled to 55°C, and then the temperature was increased by 0.5°C/sec up to 95°C. Bovine glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene, and all gene expression levels were quantified and reported relative to GAPDH expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Real-time RT-PCR was performed in duplicate for each sample/primer set.

Statistical Analysis. Data were analyzed with PROC MIXED in SAS 9.2 (SAS Inst., Inc., Cary, NC) using RFI class (high vs. low efficiency) as a fixed effect. Means were separated using LSD and were considered significant when $P \leq 0.05$. Additionally, the CORR procedure of SAS was used to determine the relationship of G:F and intake with angiogenic factor mRNA expression.

Results

It has previously been reported that small intestinal mass (g/kg BW) was less (7.63 vs. 8.50 ± 0.28 g/kg BW; $P = 0.04$) in high efficiency compared with low efficiency steers (Meyer et al., 2013). While efficiency in these finishing steers has been associated with differences in small intestinal mass, jejunal mRNA expression of *VEGF* ($P = 0.14$), *FLT* ($P = 0.58$), *KDR* ($P = 0.40$), *NOS3* ($P = 0.45$), and *GUCY1B3* ($P = 0.47$) was unaffected by RFI class (Table 2).

Partial correlation coefficients between G:F and intake during finishing and mRNA expression of angiogenic factors in the jejunum are shown in Table 3. In this study, there was no correlation of angiogenic factor expression with G:F or intake ($P \geq 0.16$).

Discussion

Although mRNA expression of the *VEGF* and NO systems did not appear to affect feed efficiency in the current study, angiogenic factor mRNA expression and vascularity measures in the jejunum have been associated with feed intake in previous studies. In agreement with the data presented here, RFI and G:F were not correlated with histological measures of vascularity and jejunal mRNA expression of these angiogenic factors in a previous cattle study (Meyer et al., 2012a; Cunningham et al., 2013). Conversely, in another study from our laboratory, jejunal mRNA expression of *FLT1* tended to be greater in more efficient lambs (Clarkson et al., 2013). It has been previously reported that nutritional plane (60, 100, or 140% of NRC recommendations) affected mRNA expression of *VEGF*, *FLT1*, *KDR*, and *NOS3* in gestating ewes (Neville et al., 2010; Meyer et al., 2012c). Additionally, total small intestinal vascularity, as well as jejunal *KDR* and *NOS3* expression, was positively correlated with intake in finishing cattle (Meyer et al., 2012a; Cunningham et al., 2013). This suggests that small intestinal gene expression of the VEGF and NO systems have a stronger relationship with feed intake than with feed efficiency.

Previous work in our laboratory has demonstrated that intestinal mass and mucosal density are associated with feed efficiency in finishing cattle (Meyer et al., 2012b). Blood flow in the small intestine is necessary for both tissue growth and nutrient transport. Thus, we can predict that

blood flow may influence efficiency in not only the small intestine, but also the whole animal. Although the current data show that relative expression of these angiogenic factors did not change with RFI class, perhaps other measures of angiogenesis and vascularity may be related to RFI in ruminants. Future research is planned to investigate histological vascularity measures, as well as additional factors related to angiogenesis, growth indices, and nutrient transporters of these small intestinal tissues.

In summary, results of this study suggest that feed efficiency is not affected by mRNA expression of the VEGF or NO systems in the small intestine of finishing steers. Further research is necessary to explore the possible relationship of small intestinal blood flow and feed efficiency.

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Table 1. Sequence of primers used for bovine angiogenic factors and receptors

Gene of interest	Description	Forward primer	Reverse primer
VEGF	Vascular endothelial growth factor	TCACCAAAGCCAGCACATAG	GCGAGTCTGTGTTTTTGCAG
FLT1	VEGF receptor-1	GTATCACTGCAAAGCCAGCA	AGCGTTAACAGGAGCCAGAA
KDR	VEGF receptor-2	CCCTTCTTTGAAGCATCAGC	CGTGCTGTTCTTCTTGGTCA
NOS3	Endothelial nitric oxide (NO) synthase	GTGGAGATCAACCTGGCTGT	GACCATCTCCTGGTGGAAGA
GUCY1B3	Soluble guanylate cyclase, binds NO	GAGGATGCCTCGCTACTGTC	CTGCTCCGTTTCCTCTGTTC

Table 2. Effects of residual feed intake (RFI) classification on relative jejunal mRNA expression of angiogenic factors in finishing steers

	RFI class[2]			
Gene of interest[1]	Low efficiency	High efficiency	SEM	*P*-value
VEGF	1.51	1.30	0.10	0.14
FLT1	2.28	2.05	0.29	0.58
KDR	2.44	2.10	0.28	0.40
NOS3	2.07	1.80	0.24	0.45
GUCY1B3	1.98	2.41	0.42	0.47

[1]*VEGF* = vascular endothelial growth factor, *FLT1* = VEGF receptor-1, *KDR* = VEGF receptor-2, *NOS3* = endothelial nitric oxide synthase 3, and *GUCY1B3* = soluble guanylate cyclase.
[2]Low efficiency = 20% highest RFI steers (n = 8); High efficiency = 20% lowest RFI steers (n = 8).

Table 3. Partial correlation coefficients of jejunal angiogenic factor mRNA expression with G:F and feed intake in finishing steers

	Relative jejunal mRNA expression[1]				
Trait	*VEGF*	*FLT1*	*KDR*	*NOS3*	*GUCY1B3*
G:F	-0.01	0.11	0.13	-0.13	0.34
	(*P* = 0.97)	(*P* = 0.70)	(*P* = 0.63)	(*P* = 0.64)	(*P* = 0.19)
Feed intake, kg	0.35	0.33	0.32	0.37	-0.08
	(*P* = 0.19)	(*P* = 0.22)	(*P* = 0.22)	(*P* = 0.16)	(*P* = 0.76)

[1]*VEGF* = vascular endothelial growth factor, *FLT1* = VEGF receptor-1, *KDR* = VEGF-receptor 2, *NOS3* = endothelial nitric oxide synthase 3, and *GUCY1B3* = soluble guanylate cyclase.

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Physiological mechanisms during variable gestation length in reindeer

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ABSTRACT: Previous reports demonstrated variable gestation length in reindeer cows. Reindeer bred early in the season have a shorter gestation than reindeer bred 30 d later ($P<0.001$). Here we report preliminary investigations of potential physiological mechanisms that could identify timing of differences in gestation. Initial objective was to explore the possibility of diapause and/or early embryonic developmental enhancement. Two groups of reindeer underwent previously established estrous synchronization protocol immediately followed by one week harem period (no other bull exposure) for early mating (Sept 1-7; n=5) or late mating (Sept 29-Oct 5; n=5). Conception was assumed to have occurred 48 to 96 hr post bull introduction as demonstrated previously. Embryos were collected 6 wk post conception and immediately weighed, measured, and developmental criteria established based on comparisons to published standards for domestic animals. They were then fixed in 10% buffered formalin, stained for cartilage (alcian blue) and bone (alizarin). Embryonic development between the two groups did not differ, providing no support for facultative diapause in the early group or embryo development enhancement in the late group. If gestation length is not established in early pregnancy, an alternative possibility is late winter when the fetus enters a phase of rapid growth. This also is associated with increasing daylength, a strong seasonal signal. The pattern of IGF-1 secretion in weekly plasma samples from pregnant (n=10) and non-pregnant reindeer (n=5) from Sept to May is highly seasonal. Mean IGF-1 concentrations decline from a Sept high (14 - 18 ng/mL) to a Dec low of 6-6.5 ng/mL until mid-Feb when IGF-1 concentrations rise rapidly to Sept levels. The rapid seasonal increase in IGF-1 is known to stimulate nutrient uptake. There is potential for this event to enhance fetal growth and contribute to variable gestation length.

Keywords: embryo, gestation, IGF-1, Reindeer

Introduction

Gestation length has generally been considered a physiologically fixed or genetic parameter (Rowell and Shipka, 2009). Despite this, estimates of gestation length in reindeer vary from 203 to 240 d, a range that is almost twice the length of the estrous cycle (Shipka *et al.* 2007). Variability in gestation length among free-ranging reindeer is commonly associated with factors such as maternal age and poor nutrition in late gestation (Reimers 2002). However, in well nourished reindeer, females bred early in the season have a longer gestation than females bred later in the fall with no difference in calf sex ratio, birth weight or calf rate of gain (Rowell and Shipka 2009). Our results are reinforced by similar experimental work in Finnish reindeer and red deer as well as numerous observational reports in a diverse range of species (see Shipka and Rowell, 2010).

While it is tempting to associate gestation variability with the ecological advantages of concentrating calving season in the spring, the mechanisms underlying such a strategy are harder to explain. If gestation variability is seasonally driven, it seems reasonable to investigate physiological events that occur when changes in daylength are maximum and provide a strong cue. In reindeer, as in most other seasonally breeding ruminants, decreasing daylength coincides with conception and very early gestation while increasing day length coincides with the phase of rapid fetal growth.

In early pregnancy, diapause or delayed implantation has the potential to extend gestation length. Reindeer don't exhibit any of the physiological characteristics of an obligate delayed implanter. However, facultative embryonic diapause remains a possibility (Tarin & Cano 1999). In fact, Ptak et al. (2012) hypothesized that embryonic diapause was a conserved phenomenon that could be induced in normally non-diapausing mammals under the right conditions.

There is also information focusing on numerous endocrine and uterine changes enhancing developmental processes during the periconception period. In particular, the role of melatonin on the ovary, the effect of increased progesterone levels, rapid trophoblast elongation, and changes to oviductal and uterine substates (see Rowell and Shipka, 2009).

In the last trimester of pregnancy when the fetus normally experiences rapid growth, mean gestation length has been extended by 10 d in New Zealand red deer by nutritional restriction (Asher *et al.* 2005a). While severe nutritional restriction in winter will lengthen reindeer gestation, the variable gestation we have documented occurred in well fed, captive reindeer. None-the-less, the period of rapid fetal growth appears to be sensitive to environmental influences.

In this report we focus on three possible mechanisms and two gestational stages; faculative diapause and developmental enhancement, both potentially occurring during the periconception period, and endocrine mediated growth of the fetus, occurring during the last trimester.

Materials and Methods

Embryo development: To address the question of facultative diapause and/or developmental enhancement, we collected 6 wk old embryos from ten mature reindeer divided into two groups, balanced for age and weight. The first group (Early; n=5) had estrus synchronized and were placed in harem for 7 d (Sept 1-7). The second group was treated identically with estrus synchronized for a late breeding (Sept 21-Sept 28) (Late; n=5). At 6 wk post-harem the females were euthanized and the embryos collected (Early group - Oct 18/19; Late group - Nov 15/16). Embryos were weighed following removal from the allantoic and amniotic membranes. Crown-rump (C-R) measurements were taken with digital calipers and fetal development compared to developmental sequences described for sheep and cattle embryos (Evans & Sack 1973). Embryos were photographed, fixed in 10% buffered formalin and stained for cartilage (alcian blue) and bone (alizarin) following the technique of Weck and Miljak (1998).

IGF-1 throughout gestation: As part of an earlier study (Shipka et al, 2007), weekly plasma samples were collected form 10 pregnant reindeer from beginning September to the end of May. They were assayed for IGF-1 using a competitive, liquid-liquid phase, double-antibody IGF-1 radioimmunoassay procedure as adapted from procedures described previously (Lalman et al, 2000) and based on the work of Holland et al., (1988) and validated for use in reindeer. Total specific binding was 39 %, the minimum detectable concentration was 1.5 ng/tube, percentage recovery of mass was > 97% across the range of 2.5 to 100uL of sample and the inter- and intra-assay CV were < 6%. Extraction recoveries for concentrations of IGF-I in 2.5-, 5-, 7.5-, 10-, 25-, 40-, and 100-ul volumes of acidified-diluted reindeer serum equaled or exceeded 99% over the 2.5 to 100ul range tested. Parallelism was assessed and verified between standard concentrations of IGF-I and the acidified-diluted serum sample containing IGF-I in volumes ranging from 2.5 to 100uL.

Results

Embryo development: In the Early group one female was not pregnant and one female had twins. In the Late group all five females had a single fetus. Seven of eight single pregnancies were in the left uterine horn and all CL of pregnancy were ipsilateral to the pregnant horn. There was no evidence of accessory CL and only one ovary contained a follicle > than 5 mm. There was no difference in fetal weight or C-R measurements in fetuses from Early or Late bred females. However, there was a difference in fetal weight (P= 0.018) and C-R length (P=0.015) between the twin fetuses and the singletons (Table 1). Following the developmental criteria of Evans & Sack (1973), external characteristics were identical in fetuses from both groups. In all cases, there were visible grooves between the fore and hind limb digits (hind limb digits were separated), tactile hair follicles could be seen surrounding the eyes and on the upper lip, the tongue was visible, the pinna covered the acoustic meatus (Figure 1). There was a genital tubercle present but sex could not be determined. This roughly corresponds with 45 d in a 280-290 d cow gestation and 34-38 d in a 147-155 d sheep gestation. The fetuses from both groups stained for cartilage but not bone following the technique of Weck & Wiljak (1998).

Table 1. Maternal body weight and age and fetal weight and crown-rump measurements in 2 groups of reindeer bred early or late in the season.

	Early (n=4)	Late (n=5)
Dam body wt prior to breeding (kg)	128.3	128.2
Dam age (yrs) (range)	6.25 (5-7)	5.40 (2-7)
Fetal wt (gms)*with twins {without twins}	*2.01 {2.34 }	2.22
Fetal C-R (cm) *with twins {without twins}	*26.99 {28.30}	28.30

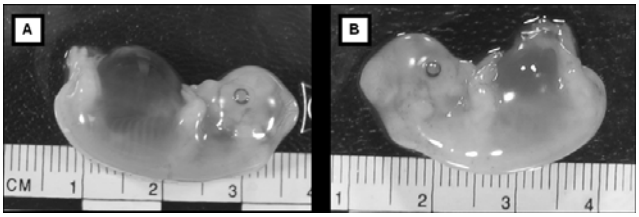

Figure 1. Representative fetuses at 6 wks gestation from the Early group (A) collected on Oct 19 and the Late group (B) collected on Nov 16/17.

IGF-1 throughout gestation: Figure 2 illustrates the pattern of systemic IGF-1 secretion and body weight from Sept to May in 10 pregnant reindeer.

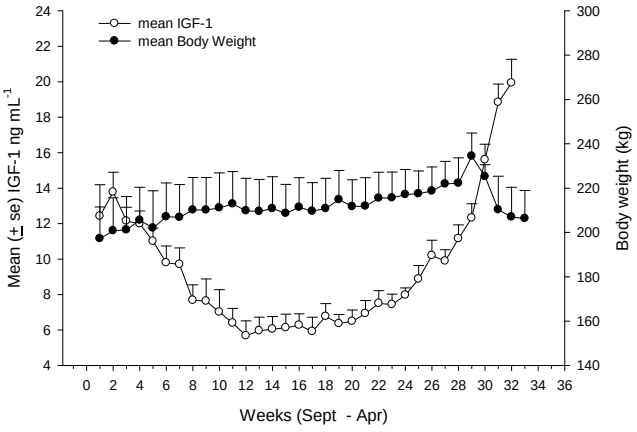

Figure 2. Seasonal pattern of IGF-1 secretion (mean ± se) and body weight (mean ± se) in 10 pregnant reindeer.

Discussion

By manipulating a single variable, time of breeding, gestation length in reindeer can be shortened or lengthened by 8-10 days. In the present study we chose to harvest embryos at 6 wk post conception in anticipation that reindeer fetal growth would be at, or near, the steepest part of the exponential growth curve making small developmental differences measureable. Further, in a different study, weekly mean P4 concentrations differed ($P < 0.001$) between early bred females and late bred females over the first 6 wk of pregnancy such that females in the late group exhibited higher P4 compared to females in the early group (Rowell and Shipka 2009). However, results of this study indicate there was no apparent difference in developmental morphology, fetal weight or C-R measurements with the exception of the twin embryos. It is interesting that even at 6 wk gestation we could detect a difference in weight and C-R between the twin and singleton fetuses even though the twin fetuses were in different uterine horns and placental connections were only just being established. The singleton pregnancies all appeared to be at approximately the same stage of gestation providing no support for facultative diapause in the early bred group or enhanced development in the late bred reindeer.

The other gestational period subject to strong light cues is in late Feb and Mar. At this stage of gestation the fetus is entering a period of rapid body growth and is sensitive to maternal nutrient reserves as demonstrated by Asher et al, (2005a,b). Reindeer do voluntarily reduce food intake beginning in the fall, gradually drawing down on body reserves over winter until green-up in the spring (Reimers, 2002). Whether bred early or late, the reindeer fetus will enter the third trimester 1-2 months before green-up. Theoretically, a fetus from an early conception should reach the phase of rapid fetal growth when the female is in better condition than 30 d later when the late conceived fetus begins rapid growth. However, the reindeer in our studies were not nutrient restricted, showed no evidence of loss of body condition or body weight and always had ad lib access to food. In fact, the phenomenon of variable gestation reported in bison (Berger, 1992) was found to occur only in well conditioned females. An alternative consideration is the converse of nutrient restriction - enhanced nutrient uptake. Consistent with this is the seasonal pattern of IGF-1 secretion, documented in red deer (Webster et al, 1996) and reindeer, the latter study in yearling reindeer subjected to manipulated light:dark regimens (Suttie et al, 1991). We found a pronounced seasonal pattern of IGF-1 secretion in pregnant reindeer with IGF-1 reaching a nadir in mid winter and beginning a steady rise in mid-February (Figure 2), a pattern consistent in both pregnant and non-pregnant females. IGF-1 acts as a stimulus to fetal growth during the last stage of gestation (Wali et al, 2012). We propose that increasing maternal levels of IGF-1 are acting as a stimulus to fetal growth in the last trimester. If a fetus from a late conception has sufficient endocrine system competence to respond to the IGF-1 stimulus, it could initiate rapid fetal growth at an earlier developmental stage than a fetus from an early conception.

Acknowledgements

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RUMINANT AND NONRUMINANT NUTRITION

EFFECTS OF MONENSIN SODIUM AND PLANT EXTRACTS CONTAINING CINNAMALDEHYDE, CAPSICUM, AND EUGENOL ON DAYS TO PUBERTY, GAIN, PREGNANCY RATE, AND FEED EFFICIENCY IN DEVELOPING BEEF HEIFERS RECEIVING A HIGH ROUGHAGE DIET

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ABSTRACT: Our objectives were to determine the effect of monensin sodium vs. a combination of plant extracts containing cinnamaldehyde, capsicum, and eugenol on ADG, feed efficiency, pubertal onset, and subsequent conception rate following AI in yearling beef heifers. Angus heifers (n=105; initial BW 347.4 ± 29.5 kg) were utilized in a completely randomized block design for a 72 d study. Heifers were randomly distributed within 4 weight blocks, and assigned to one of 3 treatments: monensin sodium (**MON**); combination of plant extracts cinnamaldehyde, capsicum, and eugenol (**CCE**); or no feed additive (**CON**). Supplement consisted of a dried distillers grain base, and was administered at a rate of 0.7 lbs·hd^{-1}·d^{-1} as a top dress on a high forage ration. The MON premix was fed to supply 200 mg·hd^{-1}·d^{-1} monensin sodium, and the CCE premix was fed to supply 1400 mg·hd^{-1}·d^{-1} of the plant extracts. All heifers were observed daily to document behavioral estrus to define age at puberty. Pubertal onset was defined as the onset of behavioral estrus and was measured in d during the 72 d feeding period. Body weight was collected on 2 consecutive d at the beginning and end of the trial to determine initial BW, final BW, and ADG. Ort samples were collected and weighed every other d to calculate feed intake and feed efficiency. Performance measurements included ADG, DMI, G:F, and pubertal onset. There was a tendency (P = 0.06) for CON (0.53 kg·hd^{-1}·d^{-1}) and MON (0.47 kg·hd^{-1}·d^{-1}) heifers to have greater ADG compared to CCE (0.41 kg·hd^{-1}·d^{-1}). There was a tendency (P = 0.09) for CON heifers to have greater AI pregnancy rate than females that received MON (59.8% vs. 44.2%). There were no differences (P > 0.10) for DMI, G:F, or pubertal onset between treatments. These data suggest no improvement in ADG, feed efficiency, AI pregnancy rate, or d to puberty in yearling beef heifers supplemented with monensin sodium or a combination of plant extracts; capsicum, eugenol, and cinnamaldehyde.

Key Words: Average daily gain, Beef heifers, Monensin, Plant extracts, Puberty

Introduction

Financial sustainability is a priority in all beef cattle operations. Any possible increases in efficiency will increase profitability. Ionophores have been used to increase feed efficiency. However, social acceptance of ionophores has been scrutinized by the public due to their antibiotic nature. It is important to identify other alternatives to develop beef heifers that maximize feed efficiency and ADG. Our objectives were to determine the effect of monensin sodium vs. a combination of plant extracts containing cinnamaldehyde, capsicum, and eugenol on ADG, feed efficiency, pubertal onset, and subsequent pregnancy rate following AI in yearling beef heifers. Increasing average daily gain will hasten pubertal onset through increased body weight and frame size which are thresholds that must be met to achieve puberty (Wiltbank et al., 1966). Supplementing Monensin increases feed efficiency in cattle (Depenbusch et al., 2008), by altering rumen microbial population to favor proprionic acid producing organisms. (Davis and Erhart., 1976). A study conducted by Moseley et al. 1977) found that 77% of heifers that received monensin reached puberty vs. 47% of the control heifers with similar ADG. In vitro studies have indicated less microbial methane production in conjunction with monensin sodium supplementation (Bartley et al., 1979). Numerous studies have shown that monensin sodium reduces rumen degradation of protein, allowing for a greater proportion of protein to "bypass" and be absorbed in the small intestine (Chen et al., 1991). Few studies have been conducted analyzing the effects of plant extracts such as cinnamaldehyde, capsicum, and eugenol on feedlot performance, and of these studies results have been conflicting. A mixture of cinnamaldehyde and eugenol have shown to reduce acetate levels and increase propionate, while reducing the levels of ammonia (Cardoza et al., 2005). When examined *In Vitro* (Cardozo et al. 2005) found that cinnamaldehyde and capsicum reduced NH_3-N concentrations, while capsicum and eugenol concurrently decreased branched chain volatile fatty acid concentrations.. Limited literature has been published examining the long term effects of monensin and these 3 plant extracts on pregnancy rates and pubertal onset in beef heifers. Therefore, the hypothesis that and a combination of the plant extracts cinnamaldehyde, capsicum, and eugenol will increase feed efficiency and gain in beef heifers, which will allow females to reach puberty at a younger age and increase pregnancy rates following TAI when compared to monensin.

Materials and Methods

Animals and Diets

One-hundred-five Angus heifers with an initial BW of 347.4 ± 29.5 kg were used in a 72-d randomized complete-block design experiment. Body weight was collected on 2 consecutive d at the beginning and end of the

trial to determine initial BW, final BW, and ADG. Based on the initial BW heifers were blocked into five different weight categories: light (273kg), mid light (297kg), medium (313 kg), mid heavy (327 kg), and heavy (354 kg). Pens were then randomly assigned to 1 of 3 treatments: monensin sodium (MON); combination of plant extracts cinnamaldehyde, capsicum, and eugenol (CCE); or no feed additive (CON). Each treatment consisted of 5 pens, with approximately 10 replicates per pen.

Supplement consisted of a dried distillers grain base, and was administered at a rate of 0.7 lbs•hd^{-1}•d^{-1} as a top dress on a high forage ration. The MON premix was fed to supply 200 mg•hd-1•d-1 monensin sodium, and the CCE premix was fed to supply 14 g•hd^{-1}•d^{-1} of the plant extracts. Heifers received supplementation from d 1 to d 72 of the feeding period; the supplement was administered directly after receiving the basal diet (Table 1).

Data Collection

Orts and as-fed samples of the ration were collected every other d, and feed refusals were collected daily and determined by a trained bunk reader. Feed was then adjusted accordingly to minimize refusals. Orts and as-fed samples of the ration were composited by both treatment and month for analysis of DM and nutrient content. The DM of these samples was determined by drying at 60°C for 48 h in a forced-air oven. Nutrient analysis was determined by SDK (SDK Laboratories, Hutchinson,KS). All heifers were observed daily to document behavioral estrus to define age at puberty. For this study pubertal onset was defined as the onset of behavioral estrus and was measured in d during the 72 d feeding period. Estrus detection aids (Estrotect Heat Detector, Rockway Inc., Spring Valley, WI) were applied to all females at the beginning of the trial and were used secondarily to visual heat detection.

Pregnancy

Heifers were stratified among treatments to 1 of 2 estrus synchronization protocols: 1) 5-d Pre-Synch + 7-d CO-Synch + CIDR, or 2) 7-d CO-Synch + CIDR. All injections were given intramuscularly at 100 µg of GnRH and 25 mg prostaglandinF2α (PGF). On d –14, the 5-d Pre-Synch heifers were administered GnRH and Controlled Internal Drug Release (CIDR), 7 d later the CIDR was removed and heifers received an injection of PGF. On d 0, all heifers received a CIDR and GnRH, followed 7 d later by PGF and CIDR removal. All heifers received TAI and GnRH 54 ±2 h after PGF. Pregnancy was diagnosed by rectal ultrasonography 55 d after TAI. Ultrasonography was conducted using a 7.5 MHz transducer.

Statistical analysis

Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC). Pen was the experimental unit as a complete randomized block design. The fixed effects were treatment as well as BW block. Response variables included DMI, ADG, Pubertal onset, and Pregnancy rate to fixed TAI. Treatment effects were defined significant at $P < 0.05$, while any trends were defined at $P \leq 0.10$

Results and Discussion

There was no difference for initial BW (347.4 ± 29.5 kg), or final BW (424.9 ± 25.1 kg; Table 2) between treatments. There was a tendency ($P = 0.06$) for CON (0.53 kg•hd^{-1}•d^{-1}) and MON (0.47 kg•hd^{-1}•d^{-1}) heifers to have greater ADG compared to CCE (0.41 kg•hd-1•d-1; Table 2). The group receiving the plant extracts had the lowest rate of gain when compared to the MON and CON heifers, while females receiving MON did not differ from the CON heifers. It has been found that cinnamaldehyde and plant extracts are dosage dependent (Busquet et al., 2005). Cattle fed a low (200 mg) and medium (800 mg) dosage level had a greater reduction in both water and feed intake than cattle fed a high (1600 mg) dosage level (Cardoza et al., 2006). These data are supported by Raun et al.,(1976); Moseley et al. (1977), which reported gains in body weight were not increased by feeding monensin. Because the CCE group received a higher than recommended dosage level (1400 mg) of plant extracts they may have had an adverse effect. This would explain the lack of increased ADG and nutrient intake which was seen in the CCE treatment group. The recommended dosage level to analyze pubertal onset and long term feedlot performance was 1100 mg of these plant extracts.

Dry matter and nutrient intakes were not different between treatments (Table 1). These data also illustrate no difference ($P > 0.05$) between treatments for gain to feed (Table 2). The lack of difference in gain to feed ratio may be assumed, because of the lack of difference in both ADG and DMI. Dry matter intake and the feed to gain ratio indicate that CCE or MON heifers were not ($P < 0.05$) more feed efficient than CON females. These results are in agreement with research conducted by Cardoza (2006), which used monensin as a positive control, and found cinnalmadehyde and monensin had a positive effect only at the beginning of the feeding period, and reported that neither monensin or the plant extract cinnamaldehyde improved long term performance throughout the entire feeding period. These data are also consistent with other research, which found that DMI was not affected between heifers fed monensin when compared to a control (Lalman et al., 1993). For this study, weights were collected at the beginning and the end of the feeding period. Therefore we cannot draw conclusion about the effects that MON and CCE had at different periods of the feeding trial. Unlike other research, these data do not suggest an increase in nutrient utilization. This may also be attributed to a high amount of refusals due to elevated levels of roughage in the diet.

No difference (P= 0.95) for pubertal onset was found between treatments (Table 3). Yet, numerically MON (88.5%) and CCE (88.5%) had a greater percent of pubertal females at the end of the study than CON (71.4%, Table 3). These results are consistent with previous research stating that the percentage of heifers pubertal at the end of the feeding period was similar for each treatment group, when comparing monensin to a control (Lalman et al., 1993). Moseley et al., (1982) stated that monensin significantly decreased pubertal onset independent of ADG or increased body weight, and it is known that body weight is a key threshold pertaining to the occurrence of pubertal onset in beef heifers (Wiltbank et al., 1966). Therefore, it has been

hypothesized, that there is a relationship between energy metabolism and responsiveness of the endocrine system with heifers fed monensin (Moseley et al., 1982). Receiving MON (30.1 d) or CCE (28.2 d) did not decrease (P=0.95) age at puberty when compared to heifers receiving CON (29.90 d; Table 2). It is important to note that the heifers utilized for this study were weaned in October and fed a high nutrient ration until December 18th, leading to hypothesize that a percentage of females were nearing an appropriate weight and age to achieve pubertal onset prior to the study. Body condition scores were taken on breeding day, no difference (P = 0.80) was found between treatments (Table 3). After the feeding period heifers were placed on grass pasture until breeding without receiving a TMR or any of the three experimental treatment supplements, this may account for the small variation and lack of difference in BCS.

Pregnancy was diagnosed 55 d after receiving fixed TAI. No difference (P=0.18) was found for pregnancy rates to TAI between treatments (Table 3). However, a tendency (P = 0.09) was found for CON heifers to have a greater pregnancy rate than females that received MON (Table 3). This is supported by Moseley et al., (1977), and McCartor et al., (1979) which showed no improvement in pregnancy rate in heifers fed monensin.

Implications

Developing heifers is a costly factor in cattle production. Beef producers must continue to seek methods to allow heifers to achieve pubertal onset with minimal inputs and increased efficiency. Further research must be conducted analyzing the effects of monensin and plant extract in order to completely examine their effects on pubertal onset.

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Table 1. Percent Dry Matter of Basal Diet

Ingredient	% DM[c]
Grass Hay	47.24
Corn Silage	33.96
Whole Corn	14.88
Dried Distillers Grain	3.90
Premix[a]	
Composition[b]	
Crude Protein	9.79
Crude Fiber	23.85
NEG	0.40
NEM	0.73
TDN	65.40
Fat	2.68
NFE	57.21

[a]Premix-Dried Distillers based supplement, mixed to supply 200 mg of monensin sodium, 1400 mg of the plant extracts cinnamaldehyde, capsicum, and eugenol, or no feed additive or ionophore.
[b]Composition-composition of the ration with a target rate of gain 0.68 kg/hd/d. NEG = Net Energy-Gain, NEM = Net Energy-Maintenance, TDN = Total Digestible Nutrients, NFE = Nitrogen Free Extract
[c]Percent dry matter of items comprising the basal ration

Table 2. The effect of monensin and plant extracts on performance in beef heifers

	Treatment[a]			
Item	MON	CCE	CON	SEM[b]
G:F	0.07	0.07	0.07	0.03
Total DMI kg	16.50	16.10	16.94	2.21
Initital BW kg	364.62	366.80	349.67	28.70
Final BW kg	431.30	421.00	422.40	25.11
ADG kg	1.08	0.90	1.17	0.39

[a]CON = Control receiving no monensin or plant extract, MON = monensin sodium group receiving 200mg/hd/d, CCE = group receiving 1400 mg of a combination of plant extracts: cinnamaldehyde, capsicum, and eugenol.

[b]Standard error of the mean.

[cd]Means within a row lacking a common superscript differ ($P < 0.05$)

Table 3. The effect of monensin and plant extracts cinnamaldehyde, capsicum, and eugenol on reproductive performance in yearling beef heifers

Item	Treatment[a]			SEM[b]
	MON	CCE	CON	
Age at Puberty d	30.07	28.18	29.90	4.70
Pubertal heifers at the end of the feeding period %	88.56	88.56	71.42	7.29
BCS at breeding	4.5	4.5	4.5	0.41
Pregnancy rate to TAI (%)	45.66	59.70	62.82	14.80

[a]Treatments:CON = Control receiving no monensin or plant extract, MON = monensin sodium group receiving 200mg/hd/d, CCE = group receiving 1400 mg of a combination of plant extracts: cinnamaldehyde, capsicum, and eugenol.

[b]Standard error of the mean.

[cd]Means within a row lacking a common superscript differ ($P < 0.05$)

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GROWTH PERFORMANCE OF RAINBOW TROUT (*Oncorhynchus mykiss*) FED ANIMAL AND PLANT PROTEIN BLEND FEEDS

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ABSTRACT: Identification of alternative protein blends that can maintain performance and reduce feed cost in rainbow trout is of great interest in aquaculture and could lead to increased long-term sustainability of the trout industry. Hence, this study investigated the performance of rainbow trout fed two experimental protein diets: animal- (APD) and plant- (PPD) based protein blends with 40% digestible protein (supplemented with lysine, methionine and threonine) compared to fish fed a 45% CP commercial trout diet (CON). A total of 675 rainbow trout with initial average BW of 125 ±3.2 g were stocked at a rate of 75 fish/2,230 L tank with three replicate tanks per diet. Fish were fed twice daily to apparent satiation in a recirculating water system for 8 weeks. Response variables were weight gain, gain per day, feed intake, and feed conversion ratio. Treatment effect was determined at $P < 0.05$ level of significance. Cost benefit-analysis also was conducted in order to determine the differences in cost of gain between dietary treatments. The results demonstrated that reducing dietary protein levels and utilizing APD or PPD protein blends in feeds did not reduce growth and feed efficiency of rainbow trout. Average fish gain and gram gain per day were not significantly different between the treatments ($P = 0.49$). Feed intake ($P = 0.55$) and FCR ($P = 0.29$) were not affected by diet. In addition, mean cost/g gain was similar in PPD and CON diets. Reducing dietary protein level, when using a plant protein blend, was demonstrated to be economically feasible in the current trial and can maintain trout production while reducing dependence on dietary fish meal inclusion.

Key words: Animal protein, growth performance, plant protein, Rainbow trout

Introduction

The high cost of feed in rainbow trout production (60% variable operating costs) has been attributed, in part, to the limited availability of marine-derived fishmeal as a dietary protein source. A typical commercial trout diet contains approximately 45% crude protein and fishmeal protein generally accounts for 25 to 50% of the dietary protein. However, because wild-harvested fishmeal stocks are finite,

research has addressed identification of appropriate alternative protein sources for rainbow trout production. Previous studies have focused on evaluating individual ingredients as replacement for fishmeal, but a more applied concept is to utilize an ideal protein blend. The use of an ideal protein concept in feed formulation has been an effective way of lowering dietary protein levels while still providing a balanced amino acid profile tailored to reflect fish muscle (Boisen et al., 2000; Gaylord and Barrows, 2009; Miles and Chapman, 2011). Effectiveness of this concept has been investigated in other livestock (Tuitoek et al., 1997) and fish nutrition studies (Ng and Hung, 1995; Gaylord et al., 2002; Furuya et al., 2004; Gaylord and Rawles, 2005), where partial or total fishmeal-free diets supplemented with the essential amino acids had the same growth performance with fishmeal diets. A caveat to this approach; however, is that sustainability of the aquaculture industry will greatly depend on identification of alternative protein blends that are comparable to fishmeal in performance and cost. Therefore, this study examined the cost/g gain of rainbow trout fed two different alternative (animal and plant-based) protein feeds as compared to a commercial rainbow trout diet.

Materials and Methods

Experimental design

The study investigated the performance of rainbow trout fed two experimental protein diets: animal- (APD) and plant- (PPD) based protein blends with 40% digestible protein (supplemented with lysine, methionine and threonine) compared to fish fed a 45% CP commercial trout diet (CON) in a feeding trial conducted at the Bozeman Fish Technology Center (BFTC) within the period of January-March, 2013. A completely randomized design was used where each of three diets were randomly allocated to three replicate tanks. Dietary effects on weight gain, feed intake, feed conversion ratio (FCR), g gain/day, survival, and cost/g gain were determined using one-way ANOVA-Proc GLM of SAS 9.1.

Experimental diets

All diets were commercially produced by Skretting North America, Toole, UT. Composition and chemical analysis of dietary treatments are provided in Table 1.

Fish culture and sampling

Rainbow trout, *Oncorhynchus mykiss***,** with initial average body weight (BW) of 125 ±3.2 g were stocked at a rate of 75 fish/2,230 L tank. Fish were fed twice daily to apparent satiation in a recirculating water system at 15^{o}C at the BFTC for period of eight weeks. Feed intake was monitored weekly and bulk tank weights were determined at four week intervals. All fish handling procedures followed the guidelines of the US Fish and Wildlife Service.

Results

Fish growth, feed utilization and survival were not significantly affected by dietary treatment (Table 2). Average 8-week weight gain per fish was 235 g for fish fed

Table1: Ingredients and chemical compositions of dietary treatments

Ingredients g/100g as fed basis	40% (APD^+)	40% (PPD^+)	45% (CON)
Poultry by product meal	24.28	-	
Blood meal	2.97	-	
Feather meal	2.95	-	
Millrun	3.18	-	
Soy isolate	-	16.88	
Corn gluten meal	8.8	21.61	
Soybean meal	12.39	15.96	
Whole wheat	20.62	15.76	
Poultry fat	7.56	10.34	
Fish oil	7.45	7.49	
Lecithin	0.93	0.94	
L-Lysine HCl	2.67	2.94	
DL Methionine	0.67	0.67	
Threonine	0.85	0.81	
Vitamin premix	1.43	1.45	
Astaxanthin pink	0.04	0.043	
Choline chloride	0.93	0.94	
Vitamin C	0.14	0.14	
Trace mineral premix	0.93	0.94	
Monocalcium phosphorus	1.21	3.09	
Analysed composition (as fed)			
Crude protein (N*6.25) (g/kg)	400±1.8	387±0.95	456±0.021
Fat (g/Kg)	142.2±2.5	205.3±3.5	169.3±0.4
Energy (kJ/kg)	21.45	21.80	21.87

CON, 229 g for fish fed APD and 235 g for fish fed PPD. Gram gain per day ranged from 4.1 g/d for fish fed APD to 4.3 g/d for fish fed PPD. Feed conversion ratios were 1.2 for fish fed CON, 1.3 for fish fed APD and 1.2 for fish fed PPD. Daily feed intake was approximately 1.7% BW/d for all treatments. Survival rates were 100% for fish fed CON, 99% for fish fed APD and 98% for fish fed PPD diets.

Table 2: Growth performance, feed utilization and cost benefit analysis

	45% CON	40% APD^+	40% PPD^+	P value	MSE
g gain[1]	235	229	241	0.4886	6.54
% BW/d[2]	185	184	191	0.7218	6.96
gain/day[3]	4.2	4.1	4.3	0.4886	0.12
FI[4]	1.7	1.7	1.7	0.5465	0.05
FCR[5]	1.2	1.3	1.2	0.2914	0.03
Survival	100	99	98	0.0701	0.44
Cost/ g gain[6]	0.79^B	0.87^A	0.80^B	0.0447	0.02

[1]Gain = (final weight (g) − initial weight (g)) x 100 / initial weight (g).[2] %BW/d (Percent increase) = (Average final weight – Average initial weight)/ Average initial weight)*100. [3]g gain per day = (Average final weight – Average initial weight)/Feeding period. [4] FI: feed intake = g feed consumed x 100 / 100 g body mass/day. [5]FCR: feed conversion ratio = g dry feed fed / g wet weight gain. [6]Cost = (Total feed consumed *unit cost)/ Total weight gain.

In contrast, dietary treatment significantly altered cost/g gain in rainbow trout (Table 2). Fish fed PPD had the same cost/g gain as fish fed CON (0.79) while the cost/g gain was higher in fish fed APD (0.87).

Discussion

Identification of appropriate alternative protein sources for rainbow trout production has been a challenging task because most cost-effective alternative ingredients generally lack fish meal's high digestibility and balanced amino acid profile. In the current study, the alternative animal- and plant-based protein blends with 40% CP and supplemental amino acids formulated using the ideal protein concept had the same performance as the fishmeal-based commercial diet containing 45% CP. The results of this study corroborate earlier observations investigating the efficacy of the ideal protein concept for diet development in

rainbow trout (Gaylord and Barrows, 2009), hybrid striped bass (Gaylord and Rawles, 2005) and poultry (Dari et al., 2005). Similar results have previously been reported in pigs (Kerr et al., 2003) and also in poultry (Aletor et al., 2000) when protein level was reduced and essential amino acids were supplemented.

Reducing the cost of plant-based diet has been a great challenge in trout nutrition since more ingredients are needed to meet its requirement for growth and maintenance (Adelizi et al., 1998). However, this study demonstrated that a cost-effective plant protein diet is achievable by combining the ideal protein concept with synthetic amino acid supplementation. Adoption of this procedure for formulation of commercial diet in order to reduce the crude protein levels currently utilized in feeds for trout production could improve sustainability of the aquaculture industry.

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METABOLIC AND MORPHOMETRIC EFFECTS OF PSYLLIUM SUPPLEMENTATION IN HORSES GRAZING RAPIDLY GROWING COOL SEASON GRASSES[1]

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ABSTRACT: This study evaluated the effects of psyllium supplementation in horses grazing rapidly growing, cool season grasses. Eleven light-breed stock horses (7 mares, 4 geldings, age = 13.5 ± 2.5, mean ± SD) were individually confined in dry lots overnight and strip grazed for 8 h daily for 30 d. Treatment horses (n = 6) received 180 g of psyllium daily. All horses received an isocaloric protein supplement. Metabolic characteristics were evaluated by assay of glucose and insulin concentrations in blood samples collected on d 0, 8, 15, 22, and 29 at 0700, 0800, 0900, 1100, 1300, and 1500 h. Changes in morphometric characteristics were assessed by measuring BW, BCS, mean neck circumference, and tail head fat mass on d 0 and 29. Data were analyzed using a Proc Mixed model of SAS for repeated measures. Significance was accepted as $P < 0.05$. Data are presented as mean ± SD. Glucose concentrations were affected ($P < 0.05$) by treatment and day. Psyllium supplementation lowered ($P < 0.05$) glucose concentrations (112.67 ± 1.87 mg/dL and 122.79 ± 2.05 mg/dL for psyllium- and control- supplemented horses, respectively) on d 8, 15, 22 and 29. Insulin concentrations varied ($P < 0.05$) by day and the interaction between treatment and time of day. Insulin concentrations decreased over the 29-d period ($P<0.05$) for all horses. Insulin concentrations were affected by an interaction between treatment and time of day ($P < 0.05$). There were no significant differences in BW, BCS, mean neck circumference, and tail head fat mass on d 0 and 29 between psyllium- and control-supplemented horses. These results indicate that psyllium supplementation lowers systemic glucose concentrations in horses grazing cool season grasses, thus, psyllium supplementation may reduce the risk of metabolic diseases in horses consuming grass high in non-structural carbohydrate (NSC) content.

Keywords: equine, glucose, insulin, non-structural carbohydrates, psyllium,

Introduction

Digestion of **NSC** from cool season pasture grasses can result in increased adiposity, risk of insulin resistance, and laminitis in horses (Frank, 2008). Results of previous studies indicate that lowering blood glucose concentrations and increasing insulin sensitivity will reduce the risk of laminitis and associated diseases (DeLatt et al., 2010). Supplementing horses with psyllium reduces blood glucose and insulin concentrations in horses that are meal-fed (Moreaux et al., 2011). The objective of this study was to evaluate the temporal changes of glucose and insulin concentrations and morphometric characteristics associated with adiposity in psyllium-supplemented horses grazing rapidly growing cool season grasses. Specifically, we tested the hypotheses that psyllium supplementation for a 29-d period does not alter temporal concentrations of glucose and insulin in horses grazing rapidly growing cool season grasses.

Materials and Methods

Animals and Treatments

Eleven light-breed stock horses, of similar body condition, from the Montana State University Equitation Herd were used in this study. All procedures and protocols were approved by the Montana State University Agricultural Animal Care and Use Committee. Horses were stratified by sex, and assigned randomly to one of two treatments: 1) supplemented with 180 $g^{-1} \bullet hd^{-1} \bullet d^{-1}$ psyllium (n = 6) or 2) no psyllium supplementation (n = 5). All horses were fed an isocaloric ration-balancing supplement, consisting of a protein, vitamin and mineral pellet (Nutrena® Empower Balance Horse Supplement, Cargill, Minneapolis, MN). Eight ha at the Bozeman Agriculture Research and Teaching Facility were used for this study with each horse grazing approximately 0.61 ha strips, separated by electric fence. Horses were acclimated to a diet of growing cool season pasture grasses for 20 d. On the first day horses were grazed for 3 h and each day thereafter grazing time increased by 1 h/d until a total of 8 h/d was reached. The time allotment of 8 h grazing was chosen based on an industry standard (Dowler and Sicilliano, 2009). Horses were individually confined in dry lots overnight, fed their supplement at 0800 each d, and turned out to graze for 8 h/d for 30 d (June 4th to July 4th) in the individual grazing strips. Horses had free access to fresh water 24 h/d.

Forage Collection and Nutrient Content

Forage from the pasture that contained the grazing strips was collected immediately before the acclimation period and every other week during the study to be

[1]This study was supported by the Bair Ranch Foundation, Billings, MT

analyzed for nutrient content. Water-soluble carbohydrate and ethanol-soluble carbohydrate content were analyzed by Equi-Analytical Laboratories (Ithaca, NY). NSC content was determined using the following equation: NSC = 100 – CP – fat – NDF – ash (Pagan, 1994). Digestible energy was determined using the following equation: DE (kcal/kg DM) = 2,118 + 12.18 (CP%) – 9.37 (ADF%) – 3.83 (hemicellulose%) + 47.18 (Fat%) + 20.35 (NSC%) – 26.3 (Ash%) (R^2 = 0.88; Pagan, 1994). Crude protein and fat content were analyzed at the Bozeman Fish Technology Center, Bozeman, MT. Acid detergent fiber and NDF were determined using the methods of Van Soest et al. (1991). Pasture intake was determined using a previously published equation based on BW (Dowler and Sicilliano, 2009). The analyses for each collection for nutrient content were conducted immediately after the samples were dry.

Blood Sampling and Assays for Glucose and Insulin

On d -1, 7, 14, 21 and 28, each horse was fitted with a jugular venous catheter for serial blood sample collections. Two samples were collected from each horse at 0700, 0800, 0900, 1100, 1300 and 1500 on d 0, 8, 15, 22 and 29. One sample was collected into a vacutainer containing sodium fluoride and potassium oxalate for assay of plasma glucose, and the second sample was collected into a vacutainer without additives for assay of serum insulin. Each sample was placed on ice within 1 min of collection and subsequently centrifuged (1,600 x *g*) at 4˚ C. Plasma and serum from samples collected at each time from each horse were decanted and stored at -22˚C. Assay of glucose concentrations was performed in duplicate aliquots using an enzymatic, colorimetric method based on the glucose kinase reaction (Infinity glucose hexokinase kit; Thermo Scientific, Hampton, NH). Insulin concentrations were determined in duplicate aliquots using a commercially available solid-phase radioimmunoassay kit (Seimens Healthcare Diagnostics, Los Angeles, CA), which was previously validated for use in horses by Freestone et al. (1991).

Morphometric characteristics were assessed on d 0 and d 29. Body weight was measured with an electronic scale. Body condition score was recorded as the mean of three trained observers (Henneke et al., 1983). Neck circumference was measured as the mean between three measurements along a straight line from the poll to the withers (Frank et al., 2006). Tail head fat mass was measured ultrasonically 10 cm lateral to the sacral spinous processes and 11 cm crainial to the tailhead origin (Kanet et al., 1987).

Statistical Analyses

Variables associated with nutrient content of forages was analyzed using a repeated measures model using R. The strip was the experimental unit with day as the repeated measure. Model included treatment, day and the treatment by day interaction.

Glucose and insulin concentrations were analyzed separately using the Proc Mixed for repeated measures analysis of SAS (SAS, Cary, NC). The model included treatment, day, time of day, and the interactions among these variables, and compound symmetry was used for these analyses. Horse was the experimental unit, with day by time of day as the repeated measure.

Morphometric characteristics were analyzed separately using the Proc Mixed for repeated measures analysis of SAS (SAS, Cary, NC). The change in morphometric characteristics from d 0 to d 29 were analyzed using a paired t test.

Means were separated using Bonferroni's Multiple Comparison tests. Significance was accepted as $P < 0.05$. Data are presented as mean ± SD.

Results and Discussion

Non-structural carbohydrate content did not differ between strips or collection days (P > 0.05). Psyllium supplementation to horses decreased ($P<0.05$; Fig. 1) glucose concentrations on d 8, 15, 22 and 29 relative to glucose concentrations of non-supplemented horses. Glucose concentrations in all horses decreased ($P < 0.05$) over the 29-d grazing period (Fig. 2). Mean glucose concentration was 112 ± 2 mg/dL in psyllium-supplemented horses and 123 ± 2 mg/dL for non-psyllium-supplemented horses; slightly higher than that previously reported in horses grazing cool season grasses by McIntosh et al. (2007; 110 ± 2 mg/dL), Staniar et al. (2007; 100 ± 6 mg/dL), and Gordon et al. (2007; 89 ± 1 mg/dL). The differences among previous studies (McIntosh et al., 2007; Staniar et al., 2007; Gordon et al., 2007) and the present study could be due to differences in breed, use of mares and geldings, climate in different geographical areas, different assays, and (or) handling and processing of samples. The most interesting finding in the present study was that glucose concentrations decreased from June to July in all horses, despite no decrease in the NSC content of the forage. As far as we know this is the first report that shows decreasing glucose concentration over a 30-d period at the beginning of the summer. The physiological reason for this is not known, but it may be due to a continuous intake of nutrients over the 8 h period compared to feeding horses twice a day (meal fed). On the other hand, another explanation may be that the metabolism of the horses used in this study was changing relative to photoperiod. Both of these explanations need further investigation.

Insulin concentrations decreased ($P < 0.05$) in all horses from a high at d 8 to a low on d 22 (Fig. 3). It is established that insulin concentrations are primarily regulated by glucose concentrations (DeLatt et al., 2010). Therefore, the decrease in insulin concentration over these days is probably a reflection of the concurrent decrease in glucose concentrations over this same period.

There was an interaction ($P < 0.05$) between treatment and time of day for insulin concentrations (Fig. 4). Insulin concentrations increased more rapidly and to higher concentrations between 0700 and 1100 h in control-supplemented than in psyllium-supplemented horses (Fig. 4). This result appears to be a reflection of

the decreased amount of insulin needed to clear glucose from the circulation when a decreased glucose load is present (Johnson et. al., 2004).

There were no differences ($P > 0.05$) for BW, BCS, mean neck circumference, and tail head fat mass on d 0 and 29, or the change in these characteristics between d 0 and d 29, between psyllium- and control-supplemented horses.

Implications

These results indicate that psyllium supplementation lowers systemic glucose concentrations and may alter insulin sensitivity, in horses grazing cool season grasses. Psyllium supplementation may be an important management tool for reducing the risk of metabolic diseases in horses consuming grass high in NSC content.

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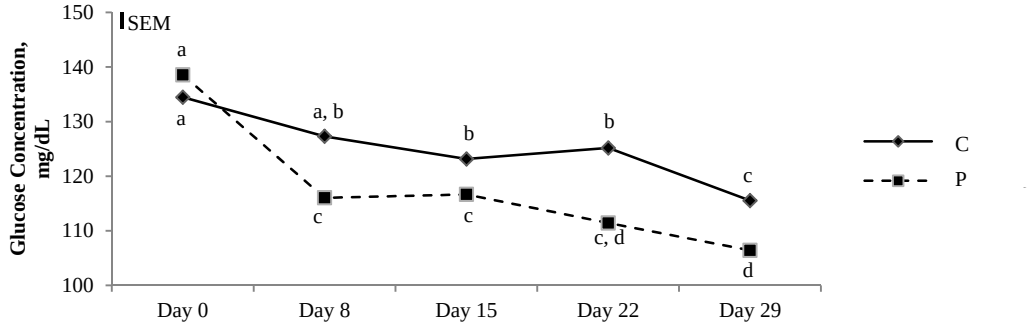

Figure 1. Mean glucose concentrations for psyllium-supplemented horses (P; 180 g^{-1}•hd^{-1}•d^{-1}; n = 6) and non-supplemented horses (C; n = 5) on d 0 (start of supplemented diets on d 1), 8, 15, 22, and 29. Pooled standard error of the mean (SEM); 3.06 mg/dL. Means with different letters differ, $P < 0.05$.

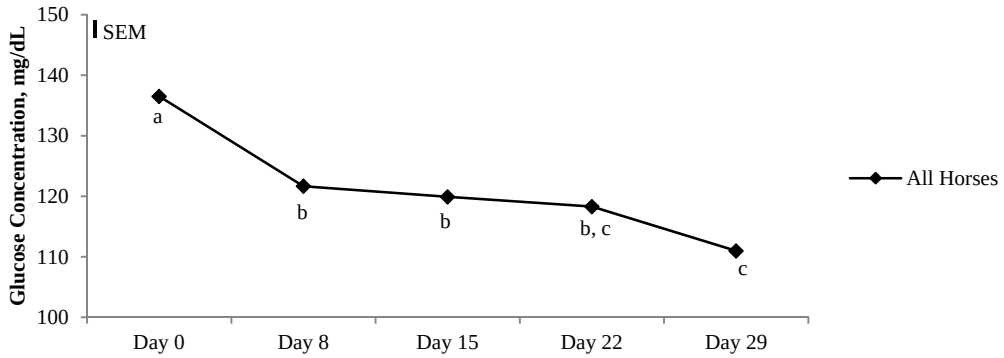

Figure 2.Mean glucose concentrations for all horses during the grazing period (n = 11) on days 0 (start of supplemented diets on day 1), 8, 15, 22, and 29. Pooled standard error of the mean (SEM); 2.82 mg/dL. Means with different letters differ, $P < 0.05$.

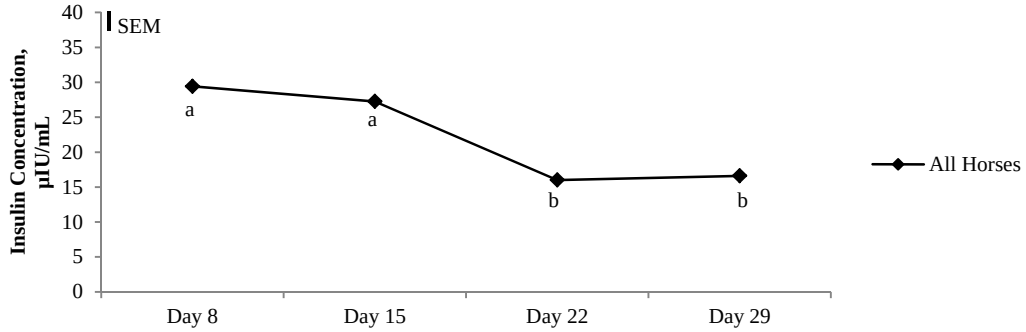

Figure 3. Mean insulin concentration for all horses (n = 11) on days 8 (start of supplemented diets on day 1), 15, 22, and 29. Pooled standard error of the mean (SEM); 2.11 µIU/mL. Means with different letters differ, $P < 0.05$.

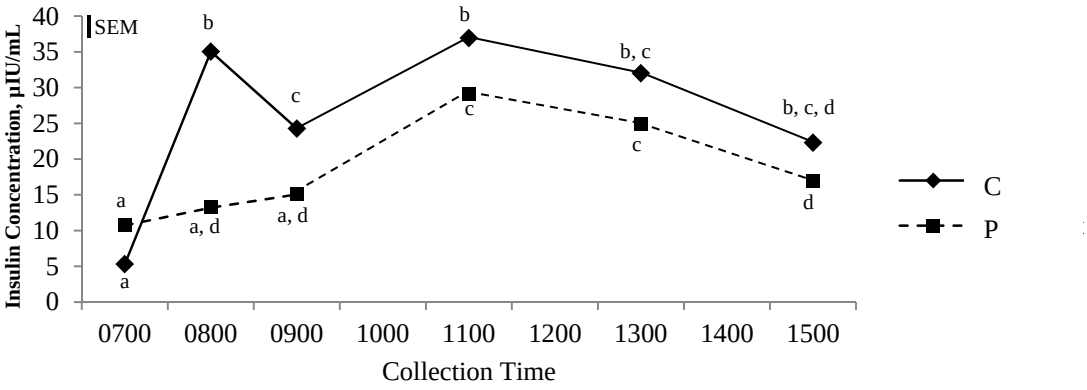

Figure 4. Mean insulin concentrations for psyllium-supplemented horses (P; 180 g^{-1}•hd^{-1}•d^{-1}; n = 6) and non-supplemented horses (C; n = 5) at times 0700, 0800 (supplement given at 0800), 0900, 1100, 1300, 1500. Pooled standard error of the mean (SEM); 3.03 µIU/mL. Means with different letters differ, $P < 0.05$.

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FEEDING LOW-PHYTIC ACID CORN GRAIN TO FINISHING WETHERS DOES NOT ALTER PHOSPHORUS DIGESTION

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ABSTRACT: The objective was to determine the extent of phytic acid degradation in sheep fed a finishing diet that was formulated with a low-phytic acid (LPA) hybrid corn grain. Crossbred wether lambs (n = 14; BW = 41.4 ± 2.5 kg), preadapted (35 d) to a finishing diet, were placed indoors (d 0) and assigned randomly and equally to 1 of 2 treatment finishing diets. Diets included ground corn grain (≈83% DM basis) from either LPA (LPG) or standard-phytic acid (SPG) hybrids. Diets were fed individually for 21 d; Cr_2O_3 was included in the diet the last 14 d. Wethers were slaughtered and contents from the duodenum and colon segments were collected. Phosphorus flows were estimated as the product of the daily Cr intake and the P:Cr concentration ratio at each segment. Chemical composition of P was determined using ^{31}P nuclear magnetic resonance spectroscopy. Total P intakes for SPG and LPG treatment groups were similar (P = 0.41) at 4,192 and 3,781 ± 315 $mg \cdot d^{-1}$ (DM basis), respectively. Inorganic phosphate was 22.1 and 76.2% and phosphate monoesters were 76.2 and 18.7% of dietary P in SPG and LPG diets, respectively. There was a net release of P from the rumen complex, which was approximately twice the daily P intake. Daily P (total and chemical forms) flows at the duodenum were similar between treatments (P = 0.42 to 0.67). Inorganic phosphate was 64.6 and 68.9% and phosphate monoesters were 34.1 and 29.0% of duodenal P flow for SPG and LPG treatments, respectively. At the colon, there was an overall minor net uptake of P. Daily P (total and chemical forms) flows at the colon were similar between treatments (P = 0.19 to 0.73). Inorganic phosphate was 79.2 and 85.2% and phosphate monoesters were 17.2 and 10.1% of colonic P flow for SPG and LPG treatments, respectively. We conclude that LPA corn grain did not improve P digestion in finishing sheep. Therefore, LPA grains may not improve efficiency of P utilization and management in ruminant production systems.

Key Words: corn, phosphorus, phytic acid, ^{31}P nuclear magnetic resonance spectroscopy

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). The USDA is an equal opportunity provider and employer.

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Introduction

Low-phytic acid (LPA) grain/oil seed hybrids contain similar concentrations of P as do standard grains and oil seeds, but the majority of P exists as inorganic phosphate rather than phytic acid (Raboy et al., 2001; Raboy, 2009). Research has shown that LPA feeds can be used to improve overall efficiency of P utilization in swine (Leytem et al., 2004), poultry (Li et al., 2001), and aquaculture (Sugiura et al., 1999) production. Unlike simple-stomached animals, ruminants can digest and utilize phytic acid, because of microbial phytases in the rumen (Morse et al., 1992; Yanke et al., 1998; Guyton et al., 2003). Nevertheless, technologies to reduce dietary phytic acid are being promoted as possible tools to improve overall P management in ruminant production systems. However, data are limiting to support such claims. Our objectives were to describe the degradation of P compounds in finishing sheep fed LPA corn grain and determine if LPA compared with standard hybrid corn grain improves P utilization in sheep.

Materials and Methods

An Institutional Animal Care and Use Committee reviewed and approved the experiment described herein.

Animals, husbandry, and diets

Fourteen crossbred (1/2 Suffolk, 1/4 Polypay, 1/4 Columbia) wether lambs (BW = 41.4 ± 2.5 kg) were weaned at approximately 120 d of age and transported 72 km to the USDA, ARS, U.S. Sheep Experiment Station Headquarters near Dubois, Idaho, USA. Lambs were placed in a feedlot pen and group-fed a series of adaptation diets (total mixed ration) over a 35-d period; grain-based concentrate was adjusted weekly, beginning at 30% and advancing to approximately 83% concentrate (DM basis). Concentrate was primarily cracked corn, and roughage was chopped alfalfa hay, which was replaced with alfalfa pellets over the course of the diet adaptation period (Table 1). Limestone, urea, NaCl, $NaHCO_3$, and a coccidiostat (lasalocid; Bovatec, Zoetis, Madison, New Jersey, USA) were included in the diet. Lambs were fed once daily, and water was provided for ad libitum intake in an automatic watering receptacle. Throughout the study, lambs were monitored twice daily for apparent ailments; if observed, ailments were treated according to type and severity.

At the end of the diet adaptation period, wethers were moved indoors and assigned randomly to individual pens (d 0). Pens were 1 m × 2.5 m and fitted with individual feed bunks and automatic watering receptacles. The facility was ventilated and cooled with an evaporative cooler; facility lighting was on continuously. Wethers were weighed and ranked by BW, and beginning with the lightest, assigned randomly to 1 of 2 finishing treatments (7 head per treatment); a detailed description of treatments is provided in the subsequent section. From d 0 to d 7, wethers were fed treatment diets twice daily at 0600 and 1800 h. Intakes were monitored daily, and amount fed was individually adjusted to allow for approximately 2 to 5% daily DM refusal. Beginning on d 8 and continuing through d 20 or 21, diets were fed 6 times daily, in equal portions, at 0200, 0600, 1000, 1400, 1800, and 2200 h. Amount fed was set at 95% of the individual maximum intake for each lamb that was determined from d 0 to 7. Starting at d 8, Cr_2O_3 was added daily to the treatment diets, which provided approximately 2.4 g $Cr_2O_3 \cdot head^{-1} \cdot d^{-1}$. If present, daily orts were collected at 0600 h, weighed, and stored. Lambs were monitored for apparent ailments, but none were observed during the experimental period.

Experimental Design, Treatments, and Sampling

A complete randomized design was use for this experiment. Treatments were finishing diets formulated with either a low-phytate (LPG) or standard corn grain hybrid (SPG). Treatment diets and corresponding nutrient analyses are presented in Table 1.

Within treatments, wethers were assigned randomly to a slaughter time on d 20 or 21. On d 20, 4 wethers from each treatment were slaughtered, and on d 21, 3 wethers from each treatment were slaughtered. Slaughter times were 0800, 1200, 1600, and 2000 h on d 20 and 0800, 1200, and 1600 h on d 21. At each time point, respective wethers received intravenous (jugular) infusions of a sodium pentobarbital solution (Beuthanasia-D Special, Schering-Plough Animal Health Corp., Union, NJ) at a rate of 80 mg of sodium pentobarbital·kg of BW^{-1}. Wethers were placed in a left lateral recumbency and exsanguinated. An incision was made parallel and caudal to the 13th rib. With minimal disturbance to intestinal tract contents, ligatures were placed throughout the digestive tract at the pyloric sphincter, 0.25 m distal to the pylorus, apparent cecum terminus, and anus. After segments were removed, duodenal and colonic contents were removed via gravity flow, tissue inversion, and(or) manual stripping. For each segment, total contents were weighed, chilled on ice (approximately 1 h), blended, divided into multiple (at least 2) aliquots, placed in polypropylene tubes, and stored at -20°C.

Sample analyses

For total P, 0.50 ± 0.02 g of lyophilized duodenum and colon samples (in duplicate) were added to 8 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 (vol/vol) and digested in a microwave oven for 2 min. Phosphorus in the digest was quantified using inductively-coupled plasma optical-emission spectrometry detection (4300DV, PerkinElmer, Wellesley, MA, USA).

Samples from 3 wethers in each treatment were selected for P chemical composition analysis, which was determined as described elsewhere (Turner, 2004) using ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR). Briefly, 2.00 ± 0.01 g of lyophilized sample (in duplicate) was added to 40 mL of 0.5 $mol \cdot L^{-1}$ NaOH and 0.05 $mol \cdot L^{-1}$ EDTA solution, agitated for 4 h at 20°C, and centrifuged at 10,000 × *g* for 30 min. Supernatant was removed and analyzed for P as described above. For ^{31}P NMR spectra, duplicate supernatants were combined, frozen rapidly (–80°C), and lyophilized. Extracts were redissolved in 0.1 mL of D_2O (for signal lock), added to 0.9 mL of 1 $mol \cdot L^{-1}$ NaOH and 0.1 $mol \cdot L^{-1}$ EDTA solution, and transferred to a 5-mm NMR tube. Spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer, operating at 202.456 MHz for ^{31}P. Analytical procedures specified were a 5-µs pulse (45°), 5.0-s delay time, 0.8-s acquisition time, and broadband proton decoupling. Number of scans varied between 3,797 and 16,091, and spectra were plotted with a line broadening of 1 Hz. Chemical shifts of signals were determined in parts per million (ppm) relative to 85% H_3PO_4 and assigned to individual P compounds or functional groups based on literature values (Turner et al., 2003a). Signal areas were calculated by integration, and P concentrations were calculated by multiplying the proportion of the total spectral area assigned to a specific signal by the total P concentration (g $P \cdot kg^{-1}$ dry feces) in the original supernatant duplicates. Detection limit of P compounds was approximately 0.1 mg $P \cdot kg^{-1}$ of dry feces (Turner, 2004).

Calculations and Statistics

Flow of total P ($mg \cdot d^{-1}$) at the duodenum and colon segments was estimated by multiplying the P:Cr concentration ratio at the respective segment by the daily Cr intake. Flow of P (mg $P \cdot d^{-1}$) as various chemical forms at the duodenum and colon segments was calculated by multiplying the proportion of P that existed as inorganic phosphate, cumulative phosphate monoesters (monoester forms of P including phytic acid and the lower inositol esters of phytic acid), or phytic acid by the total P flow in the respective segment. Phosphorus flow data were analyzed using an ANOVA (Proc GLM; SAS for Windows, v. 9.2; SAS Institute Inc., Cary, NC, USA). The model included treatment as the fixed effect. A treatment effect was significant when the probability of a greater *F* test statistic was < 0.05.

Results

Daily intake and flow of P are presented in Table 2. Daily total P intake was similar between treatment groups (P = 0.41). The majority of P in the SPG treatment diets was present as phosphate monoesters, with phytic acid P accounting for approximately 56% of the total P intake. The majority of P in LPG treatment diets was in the form of inorganic phosphate, with < 10% of P present as phytic acid.

For both treatment groups, there was an overall net contribution of P from the rumen complex, with total P flow at the duodenum being more than twice the daily P intake. Duodenal P flow was similar between treatment groups (P = 0.56). Regardless of P composition differences between the diets, no treatment differences in P flow as inorganic phosphate or phosphate monoesters were detected (P = 0.42 to 0.67). Phytic acid was not detectible in duodenal samples from either treatment group. Inorganic phosphate was 64 to 68% and phosphate monoesters were 29 to 34% of total P flow at the duodenum for SPG and LPG treatments, respectively.

For both treatment groups, there was an overall slight net uptake of P at the colon. Colonic P flow was similar between treatment groups (P = 0.73). As with duodenal P flow, no treatment differences in colonic P flow as inorganic phosphate or phosphate monoesters were detected (P = 0.19 to 0.62). Phytic acid was not detectible in the colonic samples from the LPG, but was detectable in the contents from 2 of the 3 SPG wethers (mean ± SD phytic acid P flow = 323 ± 219 mg $P \cdot d^{-1}$). Inorganic phosphate was 79 to 85% and phosphate monoesters were 10 to 17% of total P flow at the colon for SPG and LPG, respectively.

Discussion

Replacement of standard corn grain with a LPA hybrid did not alter the chemical composition of postruminal P flow. These data are consistent with our previous results where a LPA hybrid barley was tested (Leytem et al., 2007). Combined, these studies clearly demonstrated that ruminal digestion is sufficient to breakdown most, if not all, dietary phytic acid. In wethers fed the standard corn grain, colonic contents from 2 of the 3 SPG wethers tested had detectible amounts of phytic acid. It is difficult to explain the presence of phytic acid in the colon, especially because the amount detected was low (< 8% of total P intake), varied greatly between the 2 wethers (CV = 67%), and was not found in all wethers, . Therefore, to suggest that dietary corn altered colonic P composition based on absence or presence of phytic acid would be speculative at this point.

Degradation of phosphate monoesters continued as the digesta moved through the intestines. In the colon digesta, the proportional amount of P that was present as phosphate monoesters was only half of that measured in the duodenal digesta. Because the shifts in P chemical composition in the duodenal and colonic digesta flow were similar, we conclude that replacement of standard corn grains with LPA hybrids does not provide any advantage that may result in improved P utilization and management in ruminant production systems. However, we do not discount the importance of LPA seed hybrids. To the contrary, our data suggest that the most appropriate and responsible use of LPA seed hybrids is in improving nutrient utilization, P digestion, and P management in swine, poultry, and aquaculture systems.

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Table 1. Ingredient and nutrient composition (DM basis) of treatment diets that were formulated with standard (SPG) or low-phytic acid (LPG) hybrid corn grains

	Treatment diets	
Ingredient, %	SPG	LPG
Standard hybrid corn grain	82.5	-
Low-phytate hybrid corn grain	-	83.8
Alfalfa pellets	12.6	11.6
Sugarbeet CSB[1]	2.5	2.2
Supplement mix[2]	2.4	2.4
Nutrient		
DM, %	88.6	88.7
CP, %	13.2	13.3
ME, Mcal·kg^{-1}	2.94	2.96
P, mg·kg^{-1}	2,815	2,785

[1]Condensed separator byproduct.
[2]Contained limestone (29%), urea (9%), sodium bicarbonate (21%), and sodium chloride (13%). Lasalocid (Bovatec, Zoetis, Madison, New Jersey, USA) was added to provide 45 to 55 mg·head^{-1}·d^{-1}.

Table 2. Chemical composition of P flow[1] through the digestive track of wethers fed finishing diets that were formulated with standard (SPG) or low-phytic acid (LPG) hybrid corn grains

	Treatment diets[2]			
Phosphorus intake[1], mg P·d^{-1}	SPG	LPG	SE	*P*-value
Total	4,192	3,781	315	0.41
Inorganic[3]	927	2,874	227	< 0.01
Monoesters[4]	3,195	706	98	< 0.01
Phytic acid	2,338	324	64	< 0.01
Duodenum P flow[1], mg P·d^{-1}				
Total	10,129	8,317	2,041	0.56
Inorganic[3]	6,542	5,732	1,313	0.67
Monoesters[4]	3,447	2,414	817	0.42
Phytic acid	[5]	[5]	-	-
Colonic P flow[1], mg P·d^{-1}				
Total	3,498	3,761	564	0.73
Inorganic[3]	2,772	3,207	570	0.61
Monoesters[4]	601	381	99	0.19
Phytic acid	[6]	[5]	-	-

[1]Phosporus chemical composition was determined using ^{31}P nuclear magnetic resonance spectroscopy. Composition is expressed as the daily flow of P.
[2]Three wethers per treatment were selected for P chemical composition analysis of duodenal and colonic contents.
[3]Inorganic phosphate
[4]Phosphate monoesters include all monoester forms of P including phytic acid and the lower inositol esters of phytic acid.
[5]Not detected.
[6]Two of 3 wethers had low detectible levels of phytic acid P; the mean ± SD flow was 323 ± 219 mg P·d^{-1}.

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EFFECTS OF DIET AND FEED EFFICIENCY ON SMALL INTESTINAL GENE EXPRESSION OF ANGIOGENIC FACTORS IN GROWING LAMBS

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ABSTRACT: We hypothesized that small intestinal vascularity and related gene expression influence whole animal efficiency in a diet-dependent manner. The objective of this study was to investigate the impact of diet type and feed efficiency status on jejunal angiogenic factor mRNA levels in growing lambs. Wethers (n = 77) of Rambouillet, Hampshire, and Suffolk breed types were randomly assigned to receive either a concentrate-based (CONC; 50% corn and 31% wheat middlings; 12.1% CP, 17.6% NDF, 2.98 Mcal ME/kg, DM basis; n = 39) or a forage-based (FOR; 67.7% alfalfa and 27.5% wheat middlings; 16.2% CP, 36.3% NDF, 2.31 Mcal ME/kg, DM basis; n = 38) pelleted diet. Individual feed intake was measured using a GrowSafe system for 49 d, and residual feed intake (RFI), a measure of feed efficiency, was calculated by subtracting expected intake from actual intake. Expected intake was calculated by regressing ADG and metabolic mid-weight on actual feed intake. The 20% most (low RFI; n = 8) and 20% least (high RFI; n = 8) efficient lambs from each diet were slaughtered. Mucosal scrapes were taken from the jejunum of each lamb (n = 32) to determine mRNA levels of vascular endothelial growth factor (*VEGF*), VEGF receptors (*FLT1* and *KDR*), endothelial nitric oxide synthase 3 (*NOS3*), and soluble guanylate cyclase (*GUCY1B3***;** nitric oxide receptor) using real-time RT-PCR. Data were analyzed using the MIXED procedure of SAS to determine the effects of diet type, RFI class (high versus low efficiency), and their interaction. There was no effect of diet type, RFI class, or their interaction on jejunal expression of *VEGF* ($P \geq 0.25$), *KDR* ($P \geq 0.28$), *NOS3* ($P \geq 0.72$), or *GUCY1B3* ($P \geq 0.38$) genes. High efficiency lambs tended to have greater ($P \geq 0.09$) jejunal *FLT1* expression than low efficiency lambs. In this study, jejunal expression of angiogenic factors was not affected by diet type in growing lambs. Although the NOS3 system does not appear to differ between high and low efficiency lambs, the VEGF system may impact whole animal efficiency through the *FLT1* receptor.

Key words: angiogenic factors, residual feed intake, small intestine

Introduction

Facing limited resources in the cattle and sheep industries, producers are working towards solutions to decrease outputs. In the sheep industry, feed accounts for 50-70% of total production costs (Nash, 1991). A way to negate some of these costs is to select animals for improved feed efficiency. Residual feed intake (**RFI**) is a measure of feed efficiency that is both moderately heritable and genetically independent of the animal's mature size (Arthur et al., 2001; Crews, 2005).

Ongoing research on RFI in ruminants includes determination of the underlying physiological mechanisms, many of which are not well understood. One that is of particular interest to our research group is the relationship of whole animal efficiency and small intestinal biology. The small intestine is the main site of postruminal nutrient absorption and is also where a large portion of nutrients are utilized due to high cellular turnover rates and metabolic activity. The jejunum has been shown to be responsive to quantity (Burrin et al., 1990; McLeod and Baldwin, 2002) and quality of diet (Jin et al., 1994). Vascularity of the jejunum has been affected by nutritional plane in sheep, where total vascularity has increased with intake (Reed et al., 2007; Meyer et al., 2012c). The vascular endothelial growth factor (**VEGF**) and nitric oxide (**NO**) systems help to regulate blood flow to tissues, and mRNA levels of these angiogenic factors in the jejunum have also been affected by nutritional plane in sheep (Neville et al., 2010; Meyer et al., 2012c).

We hypothesized that angiogenic factor gene expression of the small intestine is related to whole animal feed efficiency, as measured by RFI, in a diet-dependent manner. The objective of this study was to determine effects of diet type and RFI class on mRNA levels of angiogenic factors within the small intestine of growing lambs.

Materials and Methods

All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee.

Animals and Diets. Growing wethers (n = 77; 51.3 ± 1.2 kg) of Rambouillet, Hampshire, and Suffolk breed types were randomly allocated by BW to receive either a pelleted concentrate-based diet (**CONC**; Table 1) or a pelleted forage-based diet (**FOR**; Table 1). Lambs were acclimated to diets and the GrowSafe System over 21 d, using a 20% increase in proportion of new feed to old feed every 4 to 5 d until the diet consisted of 100% new pelleted diet ad libitum. Daily individual feed intake was measured using the GrowSafe System (GrowSafe Systems Ltd., Airdrie, AB, Canada) for the 49-d trial.

Initial and final 2-d average BW were used to calculate 49-d ADG. Residual feed intake was determined as the deviation of actual feed intake from expected intake. Expected intake was calculated by regressing ADG and metabolic mid-weight on actual feed intake (Cammack et al., 2005). Residual feed intake was used to rank wethers by efficiency, which was used to determine which animals would be slaughtered for tissue collection.

Tissue Collection. The top 20% most efficient (low RFI; n = 8) and least efficient (high RFI; n = 8) wethers from both CONC and FOR diets (total n = 32) were transported to the UW Meat Laboratory at the end of the feeding period for slaughter. The 10% highest and 10% lowest RFI ranking wethers from each diet were slaughtered on the day after the conclusion of the feed intake test; the next 10% highest and lowest RFI ranking wethers were slaughtered 5 d later. Lambs were slaughtered using standard conventional methods, and the visceral organs were removed for dissection and sample collection.

A 15-cm section of the jejunum was removed at a location adjacent to the point 10 cm caudal from the junction of the mesenteric and ileocecal veins. The jejunal luminal surface was exposed and rinsed with PBS for complete removal of digesta before mucosal tissue was removed by scraping a glass slide over the surface. Mucosal tissue (5 g) was then flash-frozen by placing it in aluminum foil on dry-ice. Frozen issues were stored at -80°C until RNA extraction.

RNA Extraction. Quantitative real-time RT-PCR was used to determine jejunal mucosal mRNA levels of *VEGF* and its receptors (VEGF receptor 1, ***FLT1*** and VEGF receptor 2, ***KDR***), as well as endothelial NO synthase 3 (***NOS3***) and the NO receptor (soluble guanylate cyclase, ***GUCY1B3***).

Frozen jejunal mucosa samples (50 to 100 mg) were placed in 1 mL of TRI reagent (Sigma Chemical Co., St. Louis, MO) and homogenized using an electronic tissue grinder (IKA Laboratories; Wilmington, NC) for 30 sec at maximum speed. The homogenate was allowed to sit at room temperature for 5 min, and 0.2 mL of chloroform was added by shaking. This was incubated at room temperature for 10 min before being centrifuged for 15 min at 12,000 x g and 4°C. The upper aqueous layer was removed and placed in a new tube containing 0.5 mL of isopropanol, and then mixed and incubated at room temperature for 10 min. The RNA was precipitated by centrifuging for 10 min at 12,000 x g and 4°C.

The RNA pellet was re-suspended in 100 µL RNase free water and further purified using the RNeasy kit (Qiagen, Santa Clarita, CA). Briefly, 350 µL of RLT buffer was added to each sample followed by 250 µL of absolute ethanol. The samples were mixed and pipetted onto RNeasy columns provided in the kit. Samples were centrifuged, and the flow through was discarded. The columns containing RNA were washed with RW1 buffer and centrifuged again. Columns were allowed to incubate for 15 min at room temperature with 80 µL DNase 1 solution (provided by the kit), followed by another wash with RW1 buffer. Columns were washed twice with RPE buffer, and the RNA was eluted in 100 µL RNase-free water. The purified RNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Denver, CO), and 2 µg aliquots were placed in 0.5 mL tubes for cDNA synthesis.

cDNA synthesis and Real-Time RT-PCR. Two micrograms of RNA (in 15 µL of nuclease-free water) was mixed with 4 µL reverse transcription buffer (5X) and 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA) in 20 µL total volume. The mixture was placed in a thermocycler (Bio-Rad Laboratories, Richmond, CA) for 5 min at 25ºC, 30 min at 42ºC, 5 min at 85ºC, and then held at 4ºC. The cDNA was diluted with 100 µL nuclease-free water and stored at -20ºC real-time RT-PCR was performed. Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) such that amplicons were approximately 150 bp in size. Primer sequences used are in Table 2. Real-time PCR was performed by mixing 10 µL of diluted cDNA with 12.5 µL of SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), 500 pmol each of forward and reverse primer, and 0.5 µL nuclease-free water in each well of a 96-well plate. Amplification was performed using the IQ5 (Bio-Rad Laboratories, Inc., Hercules, CA), and 40 cycles of 95ºC for 30 sec and 60ºC for 30 sec. Melting curve analysis was performed post-amplification to ensure the quality of PCR products as noted by the presence of a single peak. Briefly, the PCR plate was heated to 95°C for 3 min and cooled to 55°C before the temperature was increased by 0.5°C/sec up to 95°C. Ovine glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene, and all gene expression levels were quantified and reported relative to GAPDH expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Real-time RT-PCR was performed in duplicate for each sample/primer set.

Statistical Analysis. This study was analyzed as a 2 x 2 factorial in PROC MIXED of SAS 9.2 (SAS Inst., Inc., Cary, NC) with RFI class (high vs. low efficiency), diet type (CONC vs. FOR), and their interaction in the model. Means were separated using LSD and were considered significant when $P \leq 0.05$ or considered to be tendencies when $P < 0.10$. In the absence of interactions ($P > 0.10$), main effects are reported.

Results

The effects of RFI class and diet type on jejunal mRNA expression of angiogenic factors are reported in Table 3. Jejunal expression levels of *VEGF* ($P \geq 0.25$), *KDR* ($P \geq 0.28$), *NOS3* ($P \geq 0.72$), and *GUCY1B3* ($P \geq 0.38$) were unaffected by diet type, RFI class, or the diet type by RFI class interaction. Relative expression of jejunal *FLT1* tended ($P = 0.09$) to be greater in high efficiency lambs than in low efficiency lambs. Despite this, FLT1 expression was not affected by diet type or its interaction with RFI class ($P \geq 0.43$).

Discussion

In this study, diet type did not affect the expression of angiogenic factors in the jejunum of growing lambs. Expression of genes in the NOS3 system, which plays a role in vasodilation of blood vessels, did not differ between lambs that were low or high efficiency. The VEGF system appears to possibly impact whole animal feed efficiency through the FLT1 receptor, however.

In previously reported data from this study, our group reported that visceral organ size was affected to a greater extent by diet type than RFI class, where lambs on the pelleted forage diet tended ($P = 0.09$) to have greater intestinal mass compared to the lambs on the pelleted concentrate diet (Vraspir et al., 2012). Additionally,

intestinal length was not impacted by diet type ($P \geq 0.32$) or by RFI class ($P \geq 0.12$). Conversely, Meyer et al. (2012b) observed a positive correlation of RFI with small intestinal mass and a negative correlation of RFI with small intestinal mucosal density and DNA concentration in finishing cattle. This suggested that more efficient calves had less small intestinal mass, but denser intestinal mucosa.

Blood flow is necessary to carry nutrients from the gastrointestinal tract to tissues throughout the body. Two ways to increase blood flow to tissues are angiogenesis and vasodilation. Angiogenesis, the formation of new blood vessels from existing blood vessels, is stimulated by VEGF and its receptors FLT1 and KDR (Klagsbrun and D'Amore, 1996). Vasodilation, the temporary enlargement of blood vessels, is augmented by nitric oxide synthesis through NOS3 and its receptor, GUCY1B3 (Martin et al., 2001). Meyer et al. (2012a) reported that in finishing steers, KDR and NOS3 were positively correlated with feed intake, although there was no correlation of RFI or G:F with the VEGF or NO systems.

In summary, this study suggests that expression of VEGF and NOS3 system genes in the jejunum is unaffected by diet type in sheep fed a pelleted ration. It does appear that whole animal efficiency could be impacted by the VEGF system through the VEGF receptor 1; however, RFI class and diet type did not interact to effect jejunal expression of angiogenic factors. More research is needed in this area to determine further associations between feed efficiency and small intestinal blood flow in ruminants.

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Table 1. Composition of pelleted diets fed to growing lambs

Item	FOR[1]	CONC[2]
Ingredient, % DM		
Alfalfa pellet	67.7	--
Corn	--	50.2
Wheat middlings	27.5	31.0
Corn gluten	--	10.0
Cane molasses	2.50	2.50
Salt	1.34	1.76
Calcium carbonate	0.60	2.30
Dried distillers grains with solubles	--	1.00
Calcium sulfate	--	0.75
Potassium chloride	--	0.19
Trace minerals and vitamins[3]	0.34	0.36
Analyzed nutrient composition		
DM, % as fed	92.3	91.6
CP, % DM	16.2	12.1
NDF, %DM	36.3	17.6
ADF, % DM	25.1	6.6
ME, Mcal/kg[4]	2.31	2.98

[1] FOR = pelleted forage diet.
[2] CONC = pelleted concentrate diet.
[3] Includes Selenium 1600, sheep TM ORG-Zn, Flavor APF-168, Vitamin E 20000 IU/#, and CHS/PN VT-FDLT.
[4] Calculated from NRC (2007) values.

Table 2. Sequence of primers used for ovine angiogenic factors and receptors

Gene of interest	Description	Forward Primer	Reverse Primer
VEGF	Vascular endothelial growth factor	TGAGACCCTGGTGGACATCT	TATGTGCTGGCTTTGGTGAG
FLT1[1]	VEGF receptor 1	GTATCACTGCAAAGCCAGCA	AGCGTTAACAGGAGCCAGAA
KDR	VEGF receptor 2	AGCCGTTTGTTGCTTTCAGT	AGCACATGCCCACTTTAAC
NOS3	Endothelial nitric oxide (NO) synthase	GTGGAGATCAACCTGGCTGT	GACCATCTCCTGGTGGAAGA
GUCY1B3[1]	Soluble guanylate cyclase, binds NO	GAGGATGCCTCGCTACTGTC	CTGCTCCGTTTCCTCTGTTC

[1]Indicates that a bovine primer set was used.

Table 3. Effects of residual feed intake (RFI) class and diet type on mRNA expression of angiogenic factors

	RFI Class			Diet Type			*P*-values		
Gene of interest[1]	High efficiency	Low efficiency	SEM	CONC	FOR	SEM	RFI	Diet	RFI x Diet
VEGF	2.66	2.41	0.34	2.52	2.55	0.34	0.61	0.95	0.25
FLT1	4.22	2.70	0.61	3.81	3.11	0.61	0.09	0.43	0.91
KDR	15.4	10.3	3.3	11.8	13.9	3.3	0.28	0.65	0.44
NOS3	5.70	5.15	1.08	5.54	5.31	1.15	0.72	0.88	0.92
GUCY1B3	18.1	24.2	4.8	20.0	22.3	4.8	0.38	0.73	0.71

[1] *VEGF* = vascular endothelial growth factor,, *FLT1* = VEGF receptor-1, *KDR* = VEGF receptor-2, *NOS3* = endothelial nitric oxide synthase 3, and *GUCY1B3*= soluble guanylate cyclase.

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EFFECTS OF PASTURE VS. CONFINEMENT FLUSHING ON EWE BODY WEIGHT CHANGE AND NUMBER OF LAMBS BORN[1]

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ABSTRACT: Flushing is a practice of increasing nutrient intake, before and during breeding, to increase ovulation and ultimately number of lambs born (**NLB**). Two flushing experiments were conducted to evaluate NLB per ewe, and BW gain of ewes receiving 1 of 3 treatments. Treatments were: 1) ad libitum access to pea-barley hay in confinement (**CON**); 2) ad libitum access to swathed pea-barley forage in paddocks (**PAD**); and 3) ad libitum access to swathed spring wheat straw in paddocks with 0.45 kg of an 18.85%-CP supplement·ewe^{-1}·d^{-1} (**WHT**). In Exp. 1, 90 mature Targhee ewes (65.4 ± 5.8 kg BW) were used in a completely randomized design with 30 ewes assigned to treatments CON, PAD, or WHT for a 28-d experimental period. In Exp. 2, 60 mature Rambouillet ewes (61.9 ± 6.3 kg BW) were randomly allocated to treatments CON or PAD for a 14-d experiment. In each experiment there were 3 replications with 10 ewes each. At the beginning and end of the experiments, BW was recorded for all ewes after an overnight fast. The NLB per ewe was recorded at parturition. In Exp. 1, CON ewes had a greater ADG (P = 0.03) than WHT ewes (0.25 vs. 0.17 kg·ewe^{-1}·d^{-1}, respectively; SE = 0.02) but no differences ($P \geq 0.34$) were detected among treatments for NLB. In Exp. 2, ewes in the PAD treatment had a greater NLB than ewes in the CON treatment (1.58 vs. 1.42, respectively; P = 0.04; SE = 0.13). No differences were detected between treatments for ewe ADG ($P \geq 0.82$). Flushing ewes in a grazing environment resulted in either no difference, or a greater NLB, compared to flushing ewes in confinement.

Key Words: confinement, ewes, flushing, stubble, supplement, swath grazing.

Introduction

Reproductive performance is important in determining potential profitability in sheep production (Brash et al., 1994; Matos et al., 1997; Tosh and Kemp, 1994). Flushing is the practice of increasing nutrient intake, nutrient composition, or the dynamic effect that influences BW change and BCS before and during breeding. The purpose is to increase ovulation and thus number of lambs born (NRC, 2007).

The effect of flushing is influenced by ewe age, breed, stage of breeding season, body condition, and type of nutrient fed (NRC, 2007). Researchers have flushed ewes with soybean meal (Molle et al., 1997), biuret (Torell et al., 1972a), and alfalfa cubes (Meyer et al., 1973), with reported increases in ovulation rate, fecundity, and prolificacy. Dally et al. (1994) reported that flushing ewes on mature sub-clover pastures was as effective as grazing ewes on native range pastures with supplemental alfalfa pellets at the rate of 0.91 kg·ewe^{-1}·d^{-1}. However, the literature is limited on research addressing the impact of environment on flushing. Dahmen et al. (1976) reported that over a 10 yr period, lamb production in drylot-fed ewes was consistently poorer than in pasture-grazing ewes. In contrast, Torell et al. (1972b) found that range ewes fed 2.25 kg alfalfa pellets·ewe^{-1}·wk^{-1}, fed twice weekly, did not improve lambing performance compared to drylot feeding (1.82 kg alfalfa hay·ewe^{-1}·day^{-1}).

The objective of these experiments was to compare the number of lambs born (**NLB**) and BW gain of ewes flushed in two different production environments (confinement or grazing). We proposed an experimental hypothesis that ewes flushed in a grazing environment would have increased BW gain and/or NLB compared to ewes flushed in a confinement environment.

Materials and Methods

Sheep Selection and Management. All animal procedures were approved by the Montana State University Agricultural Animal Care and Use Committee (Protocol #2009-AA04). In Exp. 1, 90 mature Targhee ewes (average BW 65.44 kg ± 5.84, non-pregnant, non-lactating, 3 to 6 yr of age) were chosen at random from a band of approximately 2,000 ewes. Ewes were transported approximately 90 miles from the Bair Ranch in Martinsdale, MT on September 25, 2010 to Montana State University's Fort Ellis Experiment Station in Bozeman, MT. Ewes were held off feed and water for approximately 48 h before arriving at the Fort Ellis Experiment Station to help eliminate gut fill differences among treatments prior to determining BW. For Exp. 2, 60 mature Rambouillet ewes (61.9 ± 6.28 kg BW, non-

[1] The authors gratefully acknowledge the Bair Ranch Foundation and the Montana Agricultural Experiment Station for their financial support of this project.

pregnant, non-lactating, 3 to 6 yr of age) were selected at random from the Red Bluff Research Ranch near Norris in Madison County, MT (approximately 35 miles from Bozeman, MT). Ewes were held off of feed and water for approximately 24 h before arrival at the Fort Ellis Experiment Station, thus allowing us to record a fasted weight for each ewe. For both experiments, ewes were immediately paint branded or ear tagged for identification purposes and their fasted weights were recorded.

Exp. 1. On September 25, 2010, ewes were randomly allocated to 1 of 3 treatments, with 30 ewes in each treatment. Treatments were: 1) ewes allowed ad libitum access to pea-barley hay in confinement **(CON)**, 2) ewes allowed ad libitum access to swathed and standing pea-barley forage in paddocks **(PAD)**, and 3) ewes allowed ad libitum access to swathed and standing spring wheat straw stubble in paddocks with 0.45 kg of an 18.85% CP supplement·ewe^{-1}·d^{-1} **(WHT)** (Table 1 and 2). Confinement pens measured 40 m by 12 m. Grazing paddocks measured 91 m by 15 m for PAD and 91 m by 50 m for WHT. Ewes in the WHT treatment received their daily ration of supplement in feed buckets; 5 buckets with evenly divided rations of supplement were split between 10 ewes. The daily allocation of supplement was consumed by ewes in approximately 5 min. Because of concerns for ad libitum access to forage on October 16 to October 22, 2010, alfalfa hay was added to both CON and PAD treatments and ewes in the WHT treatment were supplemented with wheat straw. The experiment ended October 22, 2010. After a 16 h feed and water fast, BW was recorded on October 23, 2010 and the ewes were returned to the Bair Ranch and placed on alfalfa aftermath until breeding (November 1, 2010). Lambing began April 1, 2011 and the NLB for each ewe was recorded at parturition.

Exp. 2. On September 6, 2011, 60 ewes were randomly assigned to 1 of 2 treatments. Treatments were: 1) ewes allowed ad libitum access to pea-barley hay in confinement **(CON)**, and 2) ewes allowed ad libitum access to swathed and standing pea-barley forage in paddocks **(PAD)** (Table 3). The experiment ended on September 19, 2011 and ewes were weighed on September 20, 2011 after an overnight feed and water fast. Ewes were returned to the Red Bluff Research Ranch and placed on alfalfa aftermath until breeding (November 10, 2011). Lambing began April 12, 2012 and the NLB for each ewe was recorded at parturition.

In both experiments, the experimental units were pens or paddocks with 3 replications per treatment and 10 ewes per experimental unit. Ewes had ad libitum access to treatment forage, water, and a salt/mineral supplement. All treatments were formulated to meet or exceed the NRC (2007) recommendations for mature ewes at flushing to gain 0.10 kg/d.

Forage Analysis. The Van Soest et al. (1966) method and a Daisy II Incubator (ANKOM Technology Corp., Macedon, NY) were used to measure in vitro true digestibility (IVTD) of the forages and supplement using rumen fluid collected from two cannulated cows consuming similar forage. All forage samples were analyzed for N content using a Leco FP-528 Nitrogen Analyzer (Leco Corp., St. Joseph, MI) and then multiplied by 6.25 for adjustment to CP (AOAC, 2000). Samples were analyzed for NDF and ADF using the ANKOM^{200} Fiber Analyzer (ANKOM Technology Corp., Macedon, NY; Van Soest et al., 1991; Table 2 and 3).

Statistical Analysis. Data were analyzed as a completely randomized design using the Proc GLM procedures of SAS (SAS Inst., Inc., Cary, NC). The model included the effect of treatment. Response variables were NLB, final ewe BW, and ADG. Beginning ewe BW was used as a co-variable in the analysis of final BW and ADG. Means were separated using the LSD procedure when a significant *F* value was found ($P \leq 0.05$).

Results and Discussion

In Exp. 1, ewes in the CON treatment had a greater ADG ($P = 0.03$) than WHT ewes but did not differ ($P = 0.16$) from PAD ewes. No differences ($P \geq 0.34$) were detected among treatments for ending BW or NLB. In Exp. 2, ewes in the PAD treatment had a greater NLB compared to ewes in the CON treatment (1.58 vs. 1.42, respectively ;) but no differences were detected among treatments for ending BW or ADG ($P \geq 0.19$). Numerically we noted a similar response for NLB, with PAD being 1.53 and 1.58 in Exp.1 and 2, respectively, and CON being 1.45 and 1.42 in Exp. 1 and 2, respectively. The higher SE, noted in Exp. 1 compared to Exp. 2, for NLB contributed to a lack of measurable statistical difference. The reason for this higher SE is not clear.

Lambing and Ovulation Rate. Torell et al. (1972b) reported that supplementation of range ewes fed 2.25 kg alfalfa pellets·ewe^{-1}·wk^{-1}, fed twice weekly, did not improve lambing performance compared to drylot feeding (1.82 kg alfalfa hay·ewe^{-1}·day^{-1}). Drylot feeding increased the NLB per ewe present at lambing to 128% compared to 101% in the range pasture treatment. In the second year of their study, ewes with access to improved pasture for a 34-d flushing experiment had a 28.4% increase in lambing. In a similar study, Torell et al. (1972a) reported that flushing and breeding in a drylot produced more lambs than supplementing ewes on rangeland.

Dahmen et al. (1976) flushed confined ewes with 0.45 kg barley and 1.8 kg alfalfa hay·ewe^{-1}·d^{-1}, and pasture ewes were placed on fresh lush pasture for flushing. They reported opposite results from Torell et al. (1972a, 1972b), in that the NLB per ewes bred under pasture management exceeded the drylot managed ewes and was related to the respective ovulation rates. They suggested that drylot environment may cause a later breeding season. Perhaps the CON ewes in Exp. 2 experienced lower ovulation rates and, thus, a lower NLB due to their confinement environment. However, all ewes in both treatments had lambs, indicating no treatment impact on the onset of estrus.

Hulet et al. (1962) found that supplementing mature ewes with oats had a positive effect on NLB, pounds of live lamb born, and pounds of lamb weaned compared to supplementing 2 yr old ewes with the same supplement. In a study by Molle et al. (1995), ewes fed soybean meal had a greater ovulation rate than ewes fed whole corn (1.67 vs. 1.11 ova per ewe ovulating, respectively) and unsupplemented ewes (1.25 ova per ewe ovulating).

In our experiment there was no difference in NLB among the treatments in Exp. 1 (Table 4). This could partly be due to the 28-d flushing period. Hulet et al. (1962) did see increased lamb production with a 17 d flushing period, but pointed out that a flushing period greater than 17 d did not improve lambing production. Perhaps the extended flushing period allowed all treatments to reach maximum benefit for lambing production and decrease all variation between the treatments. El-Sheikh et al. (1955) found that a high level of feed increased ovulation rates but also increased embryo mortality when measured at 40 days of gestation. Foote et al. (1959) also reported an apparent detrimental effect of full-feeding on embryonic survival. In Exp. 2 (Table 5), which consisted of a 14-d flushing period, ewes in the PAD treatment had a greater NLB compared to ewes in the CON treatment (1.58 vs. 1.42, respectively, $P = 0.04$). Tribe and Seebeck (1962) found that flushing for a period of 3 wk immediately before breeding produced increased lambing percentages of 13 and 9, respectively, compared to non-flushed ewes. These increases were almost entirely due to increased twinning rates. The response to flushing was not related to the BW changes that occurred during flushing as both the flushed and non-flushed ewes lost weight.

Ewe BW. Torrell et al. (1972b) found that for every kilogram increase in the ewe BW gain during flushing, lambing percent increased about 8%. Conversely, Hulet et al. (1962) stated that the change in ewe BW during flushing was not correlated with lamb production. Our results from Exp. 1 and 2 were similar to Hulet et al. (1962). In Exp. 1, ewes in CON had a greater ADG when compared to WHT ewes, but no difference in NLB between the three treatments was observed; final BW and ADG did not affect lambing rate (Table 4). In Exp. 2, there were no differences in ewe BW or ADG even though ewes in the PAD treatment had a greater NLB compared to ewes in the CON treatment. The difference between our results and those reported by Torrell et al. (1972b) could be attributed to the diverse forage types with varying amounts of protein. Forage CP ranged from 4.66 to 12.42% in Exp. 1 and 4.97 to 6.59% in Exp. 2.

Implications

In Exp. 1, the NLB data numerically supported our hypothesis that ewes flushed in a grazing environment would have increased BW gain and/or NLB compared to ewes flushed in a confinement environment. Lack of significance for NLB in Exp. 1 may have been caused by high standard errors.

It may be more economical for producers to flush ewes with a poorer quality forage and an additional CP supplement without a detrimental effect on ovulation and ewe weight gain. Flushing ewes in a grazing environment resulted in either no difference, or a greater NLB, compared to flushing ewes in confinement. Additional research is needed to better define the optimal production environment for flushing ewes.

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Table 1. Composition of protein supplement
(As-fed; Values provided by manufacturer)

Item	Amount
Crude Protein, min	20.0%
Crude Fat, min	2.0%
Crude Fiber, max	12.0%
Calcium, min	1.5%
Calcium, max	2.0%
Phosphorus, min	0.75%
Salt, min	1.5%
Salt, max	2.0%
Selenium, min	1.5 ppm
Vitamin A, min	24,000 IU/lb
Vitamin D, min	2,400 IU/lb
Vitamin E, min	60 IU/lb

Table 2. Experiment 1. Nutritional composition of treatment forage and supplement (DM basis)

	Treatment				
Item, %	CON[1]	PAD[2]		WHT[3]	
		Standing	Swath	Wheat	Supplement
CP	11.67	8.29	12.42	4.66	18.85
ADF	21.69	25.81	29.99	58.18	14.89
NDF	43.18	48.84	54.51	74.64	29.27
DM digestibility	67.73	65.28	74.62	56.63	81.71
OM	92.09	91.74	84.45	91.05	87.03

[1] Ewes allowed ad libitum access to pea-barley hay in confinement.
[2] Ewes allowed ad libitum access to swathed and standing pea-barley forage in paddocks.
[3] Ewes allowed ad libitum access to swathed and standing spring wheat straw stubble with 0.45 kg 18.85% CP supplement·ewe^{-1}·d^{-1}.

Table 3. Experiment 2. Nutritional composition of treatment forage (DM basis)

	Treatment		
Item, %	CON[1]	PAD[2]	
		Standing	Swath
CP	6.59	4.97	5.31
ADF	28.75	39.89	34.48
NDF	50.64	60.04	60.56
DM digestibility	56.23	54.96	49.10
OM	91.69	82.40	92.04

[1] Ewes allowed ad libitum access to pea-barley hay in confinement.
[2] Ewes allowed ad libitum access to swathed and standing pea-barley forage in paddocks.

Table 4. Experiment 1. Least squares means of CON[1], PAD[2], and WHT[3], and their respective ending BW, ADG, and number of lambs born with beginning BW as a co-variable for ADG and BW

	Treatment				*P*-value		
Item	CON[1]	PAD[2]	WHT[3]	SE	CON vs. PAD	PAD vs. WHT	CON vs. WHT
Beginning BW (kg)	65.02	66.29	65.44	1.13	0.46	0.62	0.80
Ending BW (kg)[4]	70.00	71.64	70.12	1.13	0.37	0.39	0.94
ADG (kg)[5]	0.25	0.22	0.17	0.02	0.28	0.16	0.03
No. lambs born[6]	1.45	1.53	1.64	0.13	0.70	0.54	0.34

[1] Ewes allowed ad libitum access to pea-barley hay in confinement.
[2] Ewes allowed ad libitum access to swathed and standing pea-barley forage in paddocks.
[3] Ewes allowed ad libitum access to swathed and standing spring wheat straw stubble with 0.45 kg 18.85% CP supplement·ewe^{-1}·d^{-1}.
[4] Ewes were weighed after a 16 h feed and water fast.
[5] Average daily gain of ewes over 28 d experiment.
[6] Average number of lambs born per ewe; recorded at birth.

Table 5. Experiment 2. Least squares means of CON[1] and PAD[2] and their respective ending BW, ADG, and number of lambs born with beginning BW as a co-variable for ADG and BW

	Treatment			*P*-value
Item	CON[1]	PAD[2]	SE	CON vs. PAD
Beginning BW (kg)	60.76	63.15	1.06	0.19
Ending BW (kg)[3]	63.70	63.47	0.50	0.80
ADG (kg)[4]	0.12	0.11	0.04	0.82
No. lambs born[5]	1.42	1.58	0.04	0.04

[1] Ewes allowed ad libitum access to pea-barley hay in confinement.
[2] Ewes allowed ad libitum access to swathed and standing pea-barley forage in paddocks.
[3] Ewes were weighed after a 16 h feed and water fast.
[4] Average daily gain of ewes over 14 d experiment.
[5] Average number of lambs born per ewe; recorded at birth.

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VISCERAL ORGAN MASS AND JEJUNUM CELL PROLIFERATION OF LAMBS FED ALFALFA HAY, LOCOWEED, AND FEED ADDITIVES[1]

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ABSTRACT: Swainsonine toxicity causes organ damage, decreases production, and may alter digestive function of livestock consuming locoweed. Therefore, novel products are needed to improve animal tolerance to swainsonine. This study evaluated effects of 3 novel feed products on sheep visceral organ mass and jejunum cell proliferation. Forty wether lambs (39 ± 0.4 kg BW) were divided into 4 BW blocks, and randomly assigned within block to 5 treatments. Lambs were individually fed 620 g/d of alfalfa hay and 100 g/d of corn-based feed twice daily in equal portions for 20 d. Treatments were: no locoweed or feed products (**CON**); 20 g/d of locoweed (**LOCO**); 20 g/d of locoweed and 50 g/d of feed product 1 (**AK1**); 20 g/d of locoweed and 50 g/d of feed product 2 (**AK2**); 20 g/d of locoweed and 50 g/d of feed product 3 (**AK3**). Locoweed and feed products (contained bacterial cell walls, yeasts, and enzymes) replaced alfalfa hay in the diet. After a 24 h fast, 4 randomly selected lambs from each treatment were euthanized on d 21. Data were analyzed using mixed models and contrasts to compare CON with the average for treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3. Hot carcass weights were lower ($P < 0.05$) for lambs fed treatments with locoweed compared with CON. Fasting BW and HCW were lower ($P < 0.05$) for lambs fed AK2 than LOCO. Treatment did not affect ($P \geq 0.09$) carcass dressing percentage and weights (relative to fasting BW) of jejunum, total small intestine, pancreas, spleen, and heart. Weights (relative to fasting BW) of the rumen complex, duodenum, large intestine, liver, kidney, and lung were greater ($P < 0.05$), and ileum weights were smaller ($P < 0.05$) in lambs fed treatments with locoweed than CON. However, these organ weights were not different ($P \geq 0.18$) for lambs fed AK1, AK2, or AK3 compared with LOCO. Mesenteric fat was less ($P < 0.05$) in lambs fed AK2 than LOCO, and jejunum cell proliferation was greater ($P < 0.05$) in lambs fed AK1 than LOCO. Results suggest that the novel feed products evaluated in the current study did not minimize the visceral organ weight changes associated with swainsonine toxicity in sheep.

Key words: organ weight, sheep, swainsonine

INTRODUCTION

Locoweeds (*Astragalus* and *Oxytropis* spp.) are poisonous plants that contain the toxic alkaloid, swainsonine (Molyneux and James, 1982). Swainsonine inhibits intracellular α-mannosidases, causes oligosaccharides to accumulate in lysosomes, and alters the processing of glycoproteins in animal cells (Dorling et al., 1980; Kang et al., 1993). Accumulation of glycoproteins and oligosaccharides causes intracellular vacuolization in the brain, liver, kidneys, and other tissue (James et al., 1969; Orgad et al., 1985; Novikoff et al., 1985). Animals exposed to swainsonine have enlarged organs, such as the liver, heart, kidneys, spleen, and testes, when compared to their control counterparts (Dugart-Stavanja et al., 1997). The adverse effects of locoweed toxicity in livestock include neurological abnormalities, emaciation, reproductive disorders, decreased performance, and death (Molyneux et al., 1985).

Because of the adverse effect of locoweed toxicity, livestock producers could benefit from products, feed additives, or supplements that potentially increase the tolerance of animals to swainsonine. However, previous studies (Bachman et al., 1992; Stavanja et al., 1993a; Greenberg, 1994; Pulsipher et al., 1994; Richards et al., 1999; Dugart-Stavanja et al., 1997) reported little or no beneficial effects of supplementing minerals, zeolite clays, activated charcoal, anionic resin, and bentonite clays to cattle, sheep, and rats exposed to swainsonine. In contrast, preliminary data from an unpublished study indicated that feeding novel feed products containing combinations of bacterial cell walls, yeast, and enzymes could minimize the negative effects of swainsonine on rumen epithelial cells and organ weights of sheep fed locoweed. Therefore, the objective of this experiment was to evaluate the effects of three novel feed products (Agri-King Inc., Fulton, IL) on visceral organ mass and jejunum cell proliferation of wether lambs exposed to locoweed (*Astragalus allochrous*).

MATERIALS AND METHODS

Animals, Design, and Treatments

The Institutional Animal Care and Use Committee of New Mexico State University approved all experimental procedures. In a randomized complete block design, 40 wether lambs (39 ± 0.4 kg initial BW) were equally divided into 4 blocks based on their BW. Within each block, lambs were randomly assigned to 1 of 5 dietary treatments. Treatments (Table 1) were a control diet (86% alfalfa hay

[1] Authors acknowledge A. Temple and Agri-King, Inc. for supply of feed products and support with sample analysis.

and 14% corn-based supplement) fed to lambs at 1.8% of BW (as fed) for 20 d (**CON**), CON with 20 g/d locoweed replacing alfalfa hay (**LOCO**), LOCO with 50 g/d of feed product 1 replacing alfalfa hay (**AK1**), LOCO with 50 g/d of feed product 2 replacing alfalfa hay (**AK2**), and LOCO with 50 g/d of feed product 3 replacing alfalfa hay (**AK3**). The novel feed products (Agri-King Inc., Fulton, IL) consisted of combinations of bacterial cell walls, yeast, and enzymes, with rice hulls as the carrier. Locoweed (*Astragalus allochrous*) was collected in April in New Mexico and chopped (The Western Bear Cat No 5A, by Western Land Roller Co., Hastings, NE, USA) to reduce particle size. The amount of locoweed fed was calculated to supply approximately 2 mg swainsonine per kg of BW daily. Lambs were housed in individual feeding pens from d 1 to 14 for adaptation to the dietary treatments, and in metabolism crates from d 15 to 20 for urine and fecal collections to determine nutrient digestibility and N retention (data presented previously by Allataifeh et al., 2012).

Sample Collections

After lambs were fed the dietary treatments for 20 d, 5 of 10 lambs in each block were randomly selected (one lamb per treatment in each block) and removed from feed and water for 24 h before euthanasia. Fasting BW was recorded, and lambs were then euthanized using a captive bolt stunner followed by exsanguination (according to standard procedures at the New Mexico State University Meat Laboratory). Immediately after evisceration, HCW and weights of visceral organs (stomach, small intestines, large intestines, heart, lungs, liver, pancreas, spleen, kidneys) and mesenteric fat were recorded. The duodenum was identified from the pylorus to where the gastrosplenic vein entered the mesenteric vein, the jejunum was identified as the segment from the end of the duodenum to the jejunum and ileum junction, and the ileum was identified as the remaining small intestinal segment to the ileocecal junction (Soto-Navarro et al., 2004). The jejunum was sampled in 10-cm cross section from the midpoint as described previously (Swanson et al., 1999; Soto-Navarro et al., 2004) and prepared for cellular proliferation analysis (Reynolds and Redmer, 1992).

Statistical Analysis

The experiment was a randomized complete block design (4 complete blocks based on BW and date of euthanasia), and all data were analyzed using mixed models (SAS Inst. Inc., Cary, NC) with lamb as the experimental unit. The statistical model included treatment as fixed effect and block was random. Single degree of freedom contrasts were used to compare CON with the average for all other treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3. Differences among treatments were considered significant when $P < 0.05$.

RESULTS

Fasting BW tended to be lower ($P = 0.07$), and HCW were lower ($P < 0.05$) for lambs fed treatments containing locoweed compared with CON lambs (Table 2). Fasting BW and HCW were lower ($P < 0.05$) for lambs fed AK2 than LOCO. Treatment did not affect ($P \geq 0.09$) carcass dressing percentage and weights (relative to fasting BW) of jejunum, total small intestine, pancreas, spleen, and heart. Weights (relative to fasting BW) of the rumen complex, duodenum, large intestine, liver, kidney, and lung were greater ($P < 0.05$), and ileum weights were smaller ($P < 0.05$) in lambs fed treatments with locoweed than CON. However, these organ weights were not different ($P \geq 0.18$) for lambs fed AK1, AK2, or AK3 compared with LOCO. Mesenteric fat was less ($P < 0.05$) in lambs fed AK2 than LOCO, and jejunum cell proliferation was greater ($P < 0.05$) in lambs fed AK1 than LOCO.

DISCUSSION

Effects of Feeding Locoweed

Lambs with a daily exposure of approximately 2 mg swainsonine per kg of BW had greater rumen complex, duodenum, liver, kidney, and lung weights (relative to fasting BW) than CON lambs, which is consistent with increases in the relative size of visceral organs when rats were exposed to 3.7 or 7.7 mg swainsonine per kg of BW (Dugart-Stavanja et al., 1997). In the current study, feed intake was restricted at 1.8% of BW for all treatments (nutrient intakes were presented previously by Allataifeh et al., 2012). Therefore, increases in relative visceral organ mass were likely due to swainsonine toxicosis and not because of the potential effect of nutrient intake on visceral organ size. According to Stavanja et al. (1992; 1993b), increases in the relative organ size is a good toxicosis index of animals exposed to swainsonine, particularly when compared to control animals with similar feed intake. However, Stegelmeier et al. (1999) reported no effects on the organ weights of sheep exposed to 0.80 mg or less of swainsonine per kg of BW. Therefore, increases in visceral organ size appear to occur only at high exposure to swainsonine, and may be due to tissue vacuolization, protein accumulation, cell lysis, and inflammation.

Effects of Feeding Novel Product

Preliminary research (unpublished) indicated that supplementation of novel feed products containing combinations of bacterial cell walls, yeast, and enzymes minimized the negative effects of swainsonine on rumen and liver weight of sheep fed locoweed. However, these responses were not observed in the current study. Less mesenteric fat in lambs fed AK2 than LOCO could be due to dietary energy dilution, because the carrier ingredient of the novel feed product was high fiber rice hulls (Allataifeh et al., 2012).

Conclusions

Greater relative weights for the rumen complex, duodenum, liver, kidneys, and lungs of lambs fed treatments with locoweed compared with control lambs are indicative of swainsonine toxicity. The novel feed products evaluated in the current study did not minimize the visceral organ weight changes associated with swainsonine toxicity in sheep.

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Table 1. Dietary treatments fed to lambs

Item	CON	LOCO	AK1	AK2	AK3
Ingredient, g/d					
Alfalfa hay	620	600	550	550	550
Corn grain	95	95	95	95	95
Feed product[1]	0	0	50	50	50
Locoweed[2]	0	20	20	20	20
Molasses	5	5	5	5	5
Nutrient, % DM					
OM	88.8	88.5	88.5	88.5	88.8
NDF	48.4	48.7	47.7	49.9	48.1
ADF	35.8	36.2	35.8	37.6	36.2
CP	18.3	18.4	17.3	17.5	17.7
Swainsonine[3], mg/kg DM	0	149	145	150	123

[1]Novel feed products containing a combination of bacterial cell walls, yeast, and enzymes, with rice hulls as the carrier.
[2]*Astragalus allochrous* (half moon locoweed).
[3]Analyzed using the modified α-mannosidase inhibition assay as described by Taylor and Strickland (2002).

Table 2. Fasting BW, HCW, visceral organ mass, and jejunum cell proliferation of lambs after 20 d of exposure to locoweed toxicity and supplemented with novel feed additives

	Treatments[1]						Contrasts[2]			
	CON	LOCO	AK1	AK2	AK3	SEM	CON vs other	LOCO vs AK1	LOCO vs AK2	LOCO vs AK3
No of lambs	4	4	4	4	4					
Fasting BW, kg	33.4	32.9	31.6	30.5	31.9	0.76	0.07	0.26	0.04	0.39
HCW, kg	17.3	16.8	15.8	15.1	16.0	0.43	0.01	0.12	0.02	0.19
Dressing %	51.7	51.3	50.0	49.5	50.0	0.75	0.09	0.25	0.11	0.23
Visceral organs, g/kg fasting BW										
Rumen complex[3]	24.0	27.0	28.9	27.7	28.3	1.53	0.04	0.40	0.76	0.56
Duodenum	1.02	2.00	2.18	1.73	2.00	0.32	0.02	0.66	0.61	0.93
Jejunum	13.8	13.5	13.4	14.0	11.2	1.23	0.57	0.94	0.79	0.22
Ileum	0.71	0.50	0.45	0.49	0.43	0.08	0.02	0.69	0.92	0.59
Small intestine	15.5	16.0	16.0	16.2	13.7	1.24	0.95	0.98	0.90	0.22
Large intestine	12.5	14.0	14.3	15.0	13.9	0.53	<0.01	0.71	0.22	0.91
Lungs	10.9	12.9	13.1	12.7	12.0	0.58	0.01	0.86	0.82	0.30
Liver	12.9	14.2	15.2	14.6	14.8	0.48	<0.01	0.18	0.57	0.39
Kidneys	2.76	3.15	3.38	3.22	3.10	0.12	<0.01	0.21	0.70	0.75
Heart	4.97	4.52	5.06	4.98	4.98	0.27	0.78	0.18	0.24	0.25
Spleen	1.78	1.78	1.88	1.83	1.61	0.08	0.96	0.38	0.69	0.17
Pancreas	1.19	1.37	1.39	1.50	1.59	0.15	0.12	0.90	0.55	0.31
Messenteric fat	19.7	20.1	17.3	15.8	17.0	1.04	0.09	0.09	0.01	0.06
Jejunum cell prolif., %	3.70	4.07	7.67	2.92	4.23	1.31	0.43	0.04	0.51	0.93

[1]CON = basal diet of 620 g/d of alfalfa hay and 100 g/d of corn-based feed fed to lambs in equal portions twice daily (0730 and 1930) for 20 d; LOCO = 20 g/d of locoweed replaced alfalfa hay in basal diet; AK1 = 20 g/d of locoweed plus 50 g/d of feed product 1 replaced alfalfa hay in basal diet; AK2 = 20 g/d of locoweed plus 50 g/d of feed product 2 replaced alfalfa hay in basal diet; AK3 = 20 g/d of locoweed plus 50 g/d of feed product 3 replaced alfalfa hay in basal diet. Novel feed products were supplied by Agri-King Inc.
[2]Single degree of freedom contrasts were used to compare CON with the average for all other treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3.
[3]Rumen complex includes the rumen, reticulum, omasum, and abomasum.

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EFFECTS OF RUMEN PROTECTED ARGININE ON LAMB GROWTH PERFORMANCE

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ABSTRACT: Forty Rambouillet wether lambs (initial BW 37.4 ± 0.47 kg) were used in a completely randomized design to assess the influence of rumen protected Arg on growth performance. All lambs were offered 45 g·hd^{-1}·d^{-1} of alfalfa hay (18.2% CP and 63.8% TDN, DM basis) and fed a commercial lamb grower pellet (13% CP and 69.3% TDN, DM basis) at 3.6% of BW. Lambs were randomly assigned to either receive no rumen protected ARG (CON) or rumen protected Arg fed to provide 180 mg L-Arg/kg BW (ARG). Lambs were placed in pens containing 10 lambs per pen and two pens per treatment. Body weight was measured every 14 days for a total of 42 days. Lamb intake did not differ across periods ($P \geq 0.60$). Over the first 14 d, BW gain did not differ ($P = 0.73$), however, during the second 14 d period BW gain tended ($P = 0.07$) to be greater for ARG and by the third 14 d period, BW gain was greater ($P = 0.003$) for ARG compared to CON. This increase in performance over time indicates that there is an adaptation period associated with Arg and its ability to increase BW gain. Likewise, lambs fed rumen protected Arg tended to have greater ADG ($P = 0.07$) between d 14 and 28 and had greater ($P = 0.003$) ADG during the third 14 d period. Due to the lack of difference across all time periods for DM intake and an improvement in ADG, G:F tended to be greater ($P = 0.07$) and was greater ($P = 0.04$) for ARG during the second and third period, respectively. However, due to no differences being observed for all growth performance measurements during the first 14 d and mere tendencies during the second 14 d period, overall BW gain, ADG, and G:F did not differ ($P \geq 0.23$) across treatment for the 42 feeding period. In conclusion, providing rumen protected Arg at 180 mg/kg of BW increased performance of lambs after 14 d of adaptation.

Key Words: Arginine, Growth, Lambs

INTRODUCTION

Arginine is considered a semi-essential AA in ruminants. Arginine plays a significant role in many bodily tasks, serving as a precursor for proteins, nitric oxide (**NO**), polyamines, urea, glutamate, and creatine; it is one of the most versatile AA (Wu and Morris, 1998). Moreover, Arg is key in the synthesis of NO, which serves to stimulate glucose uptake, as well as glucose and fatty acid oxidation in skeletal muscles and adipose tissue (Wenjuan et al., 2006). Studies have linked Arg to an increase in performance through higher levels of IGF-1 (Davenport, 1995) and overcoming an Arg deficiency in milk-based diets (Fligger et al., 1997). In young animals, the synthesis of AA is very rapid, therefore essential AA are needed at a high level.

Nitric Oxide has been shown to improve vascular hemodynamics (Meyer et al., 2011; Saevre et al., 2011) when fed to ruminants at 180 mg of Arg/kg of BW. This could potentially increase the blood flow to key tissues including muscle. This in conjunction with NO's role in glucose uptake (Wenjuan et al., 2006) could result in increases in animal growth performance. Tan et al. (2009) reported greater muscle gains in growing-finishing pigs when Arg was supplemented. Hassan et al. (2011) reported that Awassi lambs fed formaldehyde treated Arg at either 208 or 291 mg/kg of BW had greater ADG than non-supplemented controls. However, the use of formaldehyde is strictly regulated and not a desirable option for the livestock industry (Jenkins and McGuire, 2006). Therefore, an alternative was developed by Miller (2012) that uses a combination of waxes and hydrogenated oils that provide 55% rumen protection of Arg.

We hypothesize that supplementing a novel rumen protected Arg would increase the growth performance of lambs. Therefore, our objectives were to evaluate the effects of rumen protected Arg fed at 180 mg/kg of BW to growing lambs over the course of a 42-d feeding trial.

MATERIALS AND METHODS

All animals and procedures were handled in accordance with the New Mexico State University Institutional Animal Care and Use Committee.

Forty Rambouillet wether lambs (initial BW 37.4 ± 0.47 kg) were used in a completely randomized designed experiment. Lambs were allowed free access to water and fed once daily at 0630 a basal diet of 45 g·hd^{-1}·d^{-1} alfalfa hay (18.2% CP and 63.8% TDN, DM basis) and fed a commercial lamb grower pellet (13% CP and 69.3% TDN, DM basis) at 3.6% of BW. Lambs were randomly assigned to either rumen protected Arg (55% rumen protection; Miller, 2012) fed to provide 180 mg L-Arg/kg BW (**ARG**) or no rumen protected ARG (**CON**). Ruminal protection of Arg is necessary due the high rate of ruminal disappearance (approximately 100% within 6 h; Chalupa, 1976). Levels of rumen protected Arg fed were adjusted to take into account a ruminal protection of 55% and 40% being catabolized by the small intestine (Wu, 1998). The level of

180 mg Arg/kg BW was chosen based on the research published by others in which it was shown that vascular hemodynamics in beef steers (Meyer et al., 2011) and sheep (Saevre et al., 2011) were improved. The basal diet was calculated to provide 136% of the recommended Arg requirements for growing lambs (Nolte, 2006; NRC, 2007).

Lambs were placed into 4 partially covered pens containing 10 lambs/pen and 2 pens/treatment. Pens provided enough area for 16.7 m^2/animal. The lambs were individually weighed on 2 consecutive days at the beginning and the end of the experiment and interim weights were collected every 14 d for a total of 42 d.

Statistical Analysis.

All data were analyzed as a completely randomized design using the GLM Procedure of SAS (SAS Inst. Inc., Cary, NC). The model included effect of treatment and pen was the experimental unit. Least square means were used to differentiate treatments and differences were considered significant at $P < 0.10$.

RESULTS AND DISCUSSION

Total dietary intake did not differ ($P \geq 0.60$) amongst treatments throughout the experiment (Table 1). Body weight gain and ADG did not differ ($P = 0.73$) between treatments during the first 14 d (Table 1). However, ARG had greater BW gain and ADG ($P = 0.07$) compared to CON between d 14 and 28. This enhanced performance with Arg supplementation was also observed between d 28 and 42 ($P = 0.003$). Due to the fact that intake did not differ and ADG was greater for ARG, G:F increased during d 14 to 28 ($P = 0.07$) and d 28 to 42 ($P = 0.04$) for ARG. Tan et al. (2009) reported that supplementing 110 d old barrows fed 1.0% L-Arg and a corn soybean meal-based diet increased longissimus dorsi muscle protein and overall ADG. In ruminants, Davenport et al. (1995) concludes supplemental Arg provided at 500 or 750 mg/kg BW did not increase growth as compared to an unsupplemented control, but did increase IGF-1. The disparity between the current trial and that of Davenport et al. (1995) could possibly be due to differences in rumen protection of Arg, which were not reported.

Arginine is a semi essential amino acid that is necessary for a variety of body tasks including the synthesis of nitric oxide. Growing evidence clearly indicates that dietary supplementation or intravenous administration of Arg is beneficial in improving reproductive, cardiovascular, pulmonary, renal, gastrointestinal, liver and immune functions, as well as facilitating wound healing, enhancing insulin sensitivity, and maintaining tissue integrity (Wu et al., 2009). The improvement in growth and feed efficiency agrees with Hassan et al. (2011) who reported that Awassi lambs provided continuous supplementation of protected Arg, in a concentrate ration, have increased live weight gained, feed conversion, and final BW.

Research has indicated intravenous infusion of Arg resulted in an increase of plasma GH and a marked increase in plasma insulin in sheep and cattle (Hertelendy et al., 1970). Metabolic roles of Arg include the synthesis of insulin, glucagon, GH, and NO, thereby regulating the metabolism of protein, AA, glucose, and fatty acids (Flynn et al., 2002; Jobgen et al., 2006). Moreover, NO stimulates glucose uptake by skeletal muscles and increases blood flow to insulin-sensitive tissues, promoting substrate intake (Jobgen et al., 2006).

Studies in ruminants is still lacking due to the fact that rumen microbes quickly catabolize Arg, but new techniques, such as those utilized in this study, should allow research in ruminants to expand. Although potential does exist to improve livestock growth performance, more work needs to be done to quantify Arg exact role in growth performance.

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Table 1. Effects of rumen protected arginine on lamb growth performance

	Treatments[1]			
Item	Control	ARG	SE	*P*-value
Initial BW, kg	37.2	37.6	0.47	0.55
End BW, kg	47.6	48.3	0.34	0.27
Intake, kg/d				
d 0-14	1.49	1.48	0.03	0.95
d 14-28	1.57	1.53	0.05	0.60
d 28-42	1.78	1.75	0.05	0.71
Gain, kg				
d 0-14	3.70	3.33	0.66	0.73
d 14-28	2.86	3.51	0.13	0.07
d 28-42	3.84	4.52	0.03	0.003
d 0 - 42	10.4	10.6	0.42	0.71
ADG, kg/d				
d 0-14	0.26	0.24	0.05	0.73
d 14-28	0.20	0.25	0.01	0.07
d 28-42	0.27	0.32	0.00	0.003
d 0 - 42	0.25	0.25	0.01	0.70
G:F, kg of gain:kg of feed				
d 0-14	0.18	0.16	0.03	0.72
d 14-28	0.13	0.16	0.01	0.07
d 28-42	0.15	0.18	0.00	0.04
d 0 - 42	0.15	0.16	0.00	0.23

[1]Lambs were allowed free access to water and fed once daily at 0630 a basal diet of 45 $g \cdot hd^{-1} \cdot d^{-1}$ of alfalfa hay (18.2% CP and 63.8% TDN, DM basis) and fed a commercial lamb grower pellet (13% CP and 69.3% TDN, DM basis) at 3.6% of BW. Lambs were randomly assigned to either rumen protected Arg (55% rumen protection; Miller, 2012) fed to provide 180 mg L-Arg/kg BW (**ARG**) or no rumen protected ARG (**CON**).

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USE OF OREGANO ESSENTIAL OIL ON FINISHING HAIR LAMBS

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ABSTRACT. Oregano essential oil (OEO) is considered a plant extract capable of improving animal performance in ruminants due to its Carvacrol (CAR) content. The objective was to evaluate 3 CAR levels on hair lamb body weight, dry matter intake (DMI), average daily gain (ADG) and gain efficiency (GE). Carvacrol content in OEO was 62.7%. Treatments (TRT) were (DM basis): Control 0 g/kg CAR (CON), Monensin, 33mg /kg Rumensin 200® (R), CAR1, 0.2 g of CAR/kg; CAR2, 0.3g of CAR/kg; and CAR3 0.4g of CAR/kg. Fifty crossbred hair lambs (Dorper x Pelibuey and Charolais x Pelibuey; 25 males and 25 females) were used. Average body weight (BW) was 25.5±4.3 and 26.2±3.9 kg forfemales and males, respectively. Lambs were fed ad libitum for 70 d with 80:20 concentrate:forage ratio in diet, 1.18 NEgMcal/kg, and 23.6 % of CP. Lambs were randomly assigned to TRT (n=10) and were weighed every 14 d for ADG. Dry matter intake was recorded daily and GE was estimated. Data was analyzed in a completely random design with repeated measures on time using the PROC MIXED of SAS, lamb was the experimental unit and TRT, days on feed (D), gender (GEN) and their interaction were evaluated. For BW there was no TRT, GEN or GEN×TRT effect (P>0.05) but GEN×TRT×D was significant (P<0.05), final BW was 36.1 and 38.7 kg for females and males, respectively. In DMI (kg) only GEN×TRT×D was significant (P<0.05). Dry matter intake increased linearly. Average DMI was 1.10±0.013 and 1.14±0.012 kg for females and males, respectively. Average daily gain (kg) showed differences for GEN and D (P<0.05), and there was no effect of TRT, GEN×TRT, GEN×TRT×D. Average daily gain per treatment during the test were 0.273, 0.252, 0.270, 0.253, 0.244, and 0.294, 0.310, 0.312, 0.310, and .300, for CON, R, CAR1, CAR2 and CAR3, in females and males respectively. Gender, D, and GEN×TRT×D was significant (P<0.05) for GE, while there was no effect for TRT and GEN×TRT. Average GE per TRT during the test was 4.12, 4.12, 4.21, 4.09 and 4.60; and 3.7, 3.82, 3.68, 3.59, and 3.63, for CON, R, CAR1, CAR2 and CAR3, in females and males respectively. There was no effect of the level of Carvacrol on finishing hair lamb performance fed with a high concentrate diet.

Keywords: Oregano essential oil, finishing hair lambs, carvacrol.

Introduction

Mexico has 8, 219, 383 ovine (INEGI, 2011) and their main product is finishing lambs for national consumption. High concentrate feeding systems for feedlot lambs have shown benefits improving ADG, GE and carcass traits (Villalobos, 2010). Nowadays an important consideration in intensive production systems is the use of organic additives instead of growth promoters and antibiotics including ionophores (Chaves et al., 2008).

Essential oil compounds (EOC) have been considered an alternative to replace antibiotic additives on animal production and their performance because their similar antibiotic effects (Benchaar et al., 2008). In the benefits observed in this EOC are the health benefit in animals and a friendly environmental obtaining process (Aligiannis et al. , 2001). In the principal EOC recently studied Carvacrol (one of the major active substances in Oregano essential oil) has demonstrated selective inhibition of microorganisms in rumen (Chaves et al. 2009) this may be related with animal's performance. Mexican oregano (*Lippia graveolens HBK*) is a common native plant in arid and semiarid grasslands in northern Mexico. The objective was to evaluate the Oregano Essential Oil (**Carvacrol**, **CAR**) on finishing hair lamb performance fed with a high concentrate diet.

Materials and Methods

All procedures involving animals were approved by local official techniques for animal care (NOM-O51-ZOO-1995; Universidad Autonoma de Chihuahua, 2008). This study was conducted at the Facultad de Zootecnia y Ecologia of the Universidad Autonoma de Chihuahua, Chihuahua City, Mexico. Fifty crossbred (Dorper x Pelibuey and Charolais x Pelibuey) hair lambs, 25 females and 25 males averaging 25.5±4.3kg and 26.2±3.9 kg, respectively, were used. Before the experiment lambs received ADE vitamins, 3 way clostridial vaccine and topical parasiticide. During the experiment lambs were assigned in individual pens and fed *ad libitum* (0800 h) with free access to clean water. Basal diet (**BD**) composition was 20% alfalfa hay:80 % concentrate (Table 1). Lambs were randomly assigned to 1 of 5 treatments. Carvacrol (**CAR**) content in Oregano Essential Oil (OEO) was 62.7%. Treatments (TRT) were (DM basis): **Control, BD +** 0 g/kg CAR (**CON**); **Monensin**, **BD** + 33mg /kg Rumensin 200® (**R**); **Carvacrol 1**, **BD** + 0.2 g of CAR/kg (**CAR1)**; **Carvacrol 2**, **BD** + 0.3 g of CAR/kg (**CAR2**); and **Carvacrol 3**, **BD** +

0.4g of CAR/kg (**CAR3**). The animals received a 15 d adaptation period to the diet. Dry matter intake was evaluated daily during all the experiment and lambs were weighted at the beginning and every 14 d. Dry matter intake (**DMI**) was obtained as the average of daily intake in 14 d. Average daily gain (**ADG**) was obtained dividing the average DMI between 14 d. In order to calculate gain efficiency (**GE**) average DMI was divided by average daily gain (**ADG**). Data for DMI, ADG, Final Body Weight (**FW**) and GE were analyzed with the MIXED procedure of SAS (2002) in a completely random design with repeated measurements on time, lamb was the experimental unit, and treatments, days on feed, gender and their interactions were evaluated. Initial body weight (IBW) was used as covariable.

Results and Discussion

For DMI (kg) only GEN×TRT×D was significant ($P<0.05$) and increased linearly ($P<0.05$). Average DMI was 1.10±0.013 and 1.14±0.012 kg for females and males, respectively. Similar results were also reported by Chaves et al. (2008) using weaned Canadian Arcott lambs (initial live weight 24.6±0.77 kg) fed with a control diet or supplemented with Carvacrol at the same level used in our study, CAR1.

Daily average dry matter intake (kg) for CON was 1.13±0.02 and 1.16±0.02 in female and male lambs, respectively. In the case of R it was 1.07±0.03 and 1.11±0.02 in female and male lambs, respectively, and it means a feed intake reduction of 5.3% for females and 4.3% for males. This is a common effect of monensin found when compared to control diets (Norris et al., 1986) or essential oils. All CAR treatments in female and male lambs were similar to CON, but only CAR2 showed a feed intake reduction of 7.9% ($P>0.05$) (Benchaar et al., 2006; Yang et al., 2010).

Average daily gain (ADG, kg) showed only differences for GEN and D ($P<0.05$). Average daily gain per treatment during the test were 0.273, 0.252, 0.270, 0.253, 0.244, and 0.294, 0.310, 0.312, 0.310, and .300, for CON, R, CAR1, CAR2 and CAR3, in females and males, respectively. General ADG (kg) for females was 0.258±0.008 and 0.305±0.008 for males. There was no apparent reason for the lower ADG observed in d 42 considering that DMI increased linearly ($P<0.05$), Yang et al. (2010) had similar results.

Gender, D, and GEN×TRT×D was significant ($P<0.05$) for GE. Average GE per TRT during the test was 4.12, 4.12, 4.21, 4.09 and 4.60; and 3.7, 3.82, 3.68, 3.59, and 3.63, for CON, R, CAR1, CAR2 and CAR3, in females and males, respectively. These results are a consequence of no differences in DMI and ADG; others have reported similar results (Benchaar et al., 2006; Chaves et al., 2008).

For BW only GEN×TRT×D was observed ($P<0.05$), final BW was 36.1 and 38.7 kg for females and males, respectively. Females showed less variation than males, and all treatments finished with a live body weight heavier than 35 kg that is the minimum weight for marketing lambs in Chihuahua. Similar final BW has been reached under similar conditions (Villalobos et al., 2010).

Conclusions

Supplementing a high concentrate diet with different Carvacrol levels did not affect DMI, ADG, GE, or BW. The inclusion of oregano oil despite its odor did not affect lamb performance.

Since it appears that response in animal performance is dose dependant with essential oil supplementation, we suggest to investigate higher levels of Carvacrol with high concentrate diets in finishing hair lambs.

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Table 1. Ingredients and chemical composition (DM basis) of diets of finishing hair lambs supplemented with Carvacrol

	Treatments				
Ingredients (%)	**CON**	**M**	**CAR1**	**CAR2**	**CAR3**
Rolled sorghum	36.32	36.32	36.32	36.32	36.32
Soybean meal	34.79	34.79	34.79	34.79	34.79
Alfalfa hay, full bloom	20	20	20	20	20
Molasses cane	5	5	5	5	5
Corn gluten (60% CP)	2	2	2	2	2
Calcium carbonate	0.883	0.883	0.883	0.883	0.883
Salt	0.5	0.5	0.5	0.5	0.5
Mineral Premix[1]	0.5	0.5	0.5	0.5	0.5
Carvacrol (g/Kg MS)	0	0	0.2	0.3	0.4
Monensin (ppm)	0	33	0	0	0
Calculated chemical composition (% DM basis)					
CP	23.68	23.68	23.68	23.68	23.68
ME (Mcal/Kg)	2.739	2.739	2.739	2.739	2.739
Ca	0.791	0.791	0.791	0.791	0.791
P	0.453	0.453	0.453	0.453	0.453

CON: 0g/Kg MS Carvacrol; R: 33ppm/Kg MS Monensin; CAR1: 0.2g/Kg MS Carvacrol; CAR2: 0.3g/Kg MS Carvacrol; CAR3: 0.4g/Kg MS Carvacrol.

[1] P 12%; Ca 11.5%; Mg 0.6%; Mn 2160 ppm; Zn 2850 ppm; Fe 580 ppm; Cu 1100 ppm; I 102 ppm; Co 13 ppm; Se 9 ppm; Vitamins: A 22000 UI/Kg; E 24500 UI/Kg.

Table 2. Performance of hair lambs supplemented with Carvacrol

	Treatments										
	Control		**R**		**CAR1**		**CAR2**		**CAR3**		**S.E.**
	F	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	
DMI (Kg)	1.1	1.2	1.1	1.1	1.1	1.2	1.0	1.2	1.1	1.2	0.0134
IBW (kg)	25.9	25.9	25.9	25.9	25.9	25.9	25.9	25.9	25.9	25.9	4.2
FBW (Kg)	36.8	39.5	36.4	37.9	37.1	38.3	34.8	39.1	35.5	38.8	0.3602
ADG (Kg)	0.273	0.293	0.252	0.310	0.270	0.312	0.253	0.31	0.244	0.3	0.008
GE	4.1	3.7	4.1	3.8	4.2	3.7	4.1	3.6	4.6	3.6	0.102

CON: 0g/Kg MS Carvacrol; R: 33ppm/Kg MS Monensin; CAR1: 0.2g/Kg MS Carvacrol; CAR2: 0.3g/Kg MS Carvacrol; CAR3: 0.4g/Kg MS Carvacrol.

F= female hair lambs, M= male hair lambs

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EFFECTS OF MID- TO LATE GESTATIONAL ENERGY SOURCE ON JEJUNAL MUCOSAL ANGIOGENIC FACTOR mRNA EXPRESSION IN MATURE EWES

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ABSTRACT: Small intestinal angiogenic factor expression has previously been affected in ewe models of developmental programming that result in altered ewe metabolism and fetal growth. Thus, we hypothesized that ewe jejunal mucosal mRNA expression of vascular endothelial growth factor (*VEGF*) and nitric oxide (NO) systems would be responsive to late gestational energy source, which has previously been demonstrated to elicit both dam and offspring effects. Mature Polypay ewes (n = 14; carrying either single or twin fetuses) were allocated to receive 1 of 3 diets (3.52 Mcal ME/d) with different primary energy sources from d 67 ± 3 of gestation: ad libitum alfalfa haylage (HL), limit-fed whole shelled corn (CN), or limit-fed corn dried distillers grains plus solubles (DG). Ewes fed the CN and DG diets were given trace minerals, vitamins, and additional CP (CN only) to meet or exceed NRC recommendations due to a restriction of DMI. Haylage (12.2% of the diet, DM basis) and lasalosid (27 mg/kg of dietary DM) were also fed to minimize rumen health problems in ewes fed DG and CN. On d 130 of gestation, non-survival surgery took place, followed by ewe necropsy. The small intestine was dissected and jejunal mucosa samples were collected for real-time RT-PCR of selected angiogenic factors (*VEGF*, *VEGF* receptor-1 [*FLT1*] and receptor-2 [*KDR*], endothelial NO synthase 3 [*NOS3*], and soluble guanylate cyclase [NO receptor; *GUCY1B3*]). Data were analyzed in PROC MIXED of SAS 9.2 with gestational energy source and fetal number as fixed effects. There was no effect of gestational energy source on ewe jejunal mRNA expression of *VEGF* (P= 0.97), *FLT1* (P = 0.44), *KDR* (P = 0.36), *NOS3* (P = 0.53), or *GUCY1B3* (P = 0.73). In this study, small intestinal angiogenic factor expression was not affected by prepartum diets differing in primary energy source, suggesting that vascularity of the small intestinal may not have played a role in observed dam and offspring responses in this model.

Key words: feed efficiency, gestation, small intestine

Introduction

A growing body of evidence supports the concept of developmental programming in ruminants, and we now know that poor maternal nutrition during gestation can have many lasting effects on offspring, including decreased postnatal growth and performance as well as increased morbidity and mortality (Wu et al., 2006; Caton and Hess, 2011). Not only does nutritional plane cause these effects, but recent studies demonstrate that energy source can also affect fetal and postnatal growth, even when energy requirements are met during gestation (Radunz et al., 2011a,b, 2012).

The ruminant small intestine changes in response to nutrient intake during gestation (Reed et al., 2007; Caton et al., 2009; Meyer et al., 2012b), suggesting that alterations within the small intestine contribute to observed physiological and performance changes of developmental programming. Small intestinal vascularity and angiogenic factor expression have previously been affected in gestational ewe models utilizing divergent nutritional planes that result in altered ewe metabolism and fetal growth (Neville et al., 2010; Meyer et al., 2012b). Because alternate energy sources such as limit-fed corn or dried distillers grains with solubles (**DDGS**) diets provide different nutrients (protein, fat, starch, and fiber) and DM to the small intestine compared with ad libitum forage-based diets, we hypothesized that mRNA expression of angiogenic factors in the small intestine would respond to dietary energy source during gestation. Our objectives were to investigate the effects of 3 energy sources during mid- to late gestation on the jejunal mRNA expression of vascular endothelial growth factor (***VEGF***) and nitric oxide (**NO**) systems in the ewe.

Materials and Methods

All procedures used in this experiment were approved by the University of Wisconsin-Madison (**UW**) Research Animal Resource Committee. Detailed descriptions of ewe management and non-survival surgeries have been previously described by Berg (2012).

Animal Management and Treatments. Mature Polypay ewes (n = 15; initial BW = 74.7 ± 2.5 kg) from the UW Sheep Research Unit were used in this study. Ewes were synchronized for estrus using controlled internal drug release devices (Eazi-breed CIDR, West Ryde, NSW) and naturally bred to a single sire. Before and after breeding, ewes were managed as one group and received ad libitum alfalfa haylage. Transabdominal ultrasonography was conducted 26 d post-breeding to determine pregnancy status and then again on d 33 and 37 to determine fetal number.

On d 50 of gestation, ewes were transported 32.5 km to the campus UW Livestock Laboratory. Ewes were penned and fed individually (1.2 x 2.4 m pens) in a temperature- (15.6°C) and lighting-controlled (15 h) building. Ewes were allowed 10 d to acclimate to this facility before beginning gestational treatments.

Ewes were randomly assigned to 1 of 2 rooms and stratified to treatments by BW (74.7 ± 2.5 kg), BCS (3.5 ±

0.4), ewe age (3.7± 0.9 yr), fetal age, and fetal number. Starting on d 67 ± 3 of gestation, ewes were fed 1 of 3 diets (Table 1) formulated to meet or exceed NRC (2007) nutrient requirements for mid-gestation. These diets provided 3.52 Mcal ME/d with 1 of 3 primary energy sources: ad libitum alfalfa haylage (**HL**), limit-fed whole shell corn (**CN**), or limit-fed corn DDGS (**DG**). Intake of CN and DG was limited to achieve isoenergetic intake relative to the ad libitum intake of ewes fed the HL diet. The HL diet included ad libitum intake of a trace mineral and vitamin mixture (Sheep Mineral Plus, Vita Plus Corporation, Madison, WI). Ewes fed the CN and DG diets were given the same trace mineral and vitamin mixture, as well as additional CP (CN only) to meet or exceed NRC (2007) recommendations. Haylage (12.2% of dietary DM) and lasalosid (27 mg/kg of dietary DM; Alpharma, LLC, Bridgewater, NJ) were provided to ewes fed the CN and DG diets to minimize ruminal upsets or health problems associated with feeding concentrate-based diets. Ewes were transitioned to limit-fed diets over a 7-d period. From d 116 of gestation until the end of experiment, ewes fed the HL diet were supplemented with corn gluten feed (10.36% of dietary DM) to account for late gestational energy demands of fetal growth. Diets were fed once daily at 0630 h. Ewe BW was collected weekly, and feed offered was adjusted to maintain similar BW gain for ewes fed CN and DG vs. HL.

Non-survival Surgery and Tissue Collection. On d 130 ± 1 of gestation, all ewes underwent non-survival surgery. Ewes were allowed access to their daily ration 1 h prior to being transported 2.3 km to the UW Department of Obstetrics and Gynecology, Perinatal Research Laboratories, and then were offered the remainder of their diet until 15 min prior to the start (≥ 2 h after transport) of the procedure. The non-survival surgery procedure used was similar to that described by Magness et al. (1998). After this procedure, ewes were euthanized with intravenous pentobarbital sodium (50 to 70 mg/kg BW).

Ewes were eviscerated, visceral organs were dissected, and the jejunum was sampled as described by Meyer et al. (2012b). A 15-cm jejunal sample was removed starting at a point adjacent to 10 cm caudal from the junction of the mesenteric and gastrosplenic vein on the mesenteric vein. The jejunal sample was cut open along the mesenteric side to expose the intestinal lumen and rinsed with warm PBS to remove digesta. Mucosal tissue was scraped from the jejunum using a glass slide, wrapped in aluminum foil, and flash-frozen in liquid N_2. Frozen mucosal tissues were stored at -80°C for angiogenic factor mRNA expression.

Angiogenic Factor mRNA Expression. Jejunal mucosal mRNA expression of *VEGF*, VEGF receptors (***FLT*** and ***KDR***), endothelial NO synthase 3 (***NOS3***), and soluble guanylate cyclase (***GUCY1B3***) was determined using quantitative real-time RT-PCR (Austin et al., 2011). Primer sequences used are given in Table 2. Frozen tissue was placed in 1 mL of TRI reagent (Sigma Chemical Co., St. Louis, MO) and homogenized using an electronic tissue grinder (IKA Laboratories; Wilmington, NC). The RNA pellet was resuspended in 100 µL RNase free water and further purified using the RNeasy kit (Qiagen, Santa Clarita, CA). The purified RNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Denver, CO).

Two micrograms of RNA (in 15 µL nuclease-free water) were mixed with 4 µL reverse transcription buffer (5X) and 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA). The mixture was placed in a thermocycler for 5 min at 25ºC, 30 min at 42ºC, 5 min at 85ºC, and held at 4ºC. The cDNA was diluted with 100 µL nuclease-free water and stored at -20ºC until semi-quantitative real-time PCR was performed. Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) such that amplicons were approximately 150 bp in size. Real-time PCR was performed by mixing 10 µL of diluted cDNA with 12.5 µL of SYBR green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), 500 pmol each of forward and reverse primer, and 0.5 µL of nuclease free water in each well of a 96-well plate. Amplification was performed using the IQ5 and 40 cycles, 95ºC for 30 sec and 60ºC for 30 sec, for *VEGF*, *FLT1*, and *KDR* primers. *NOS3*, and *GUCY1B3* were run for 50 cycle under the same conditions do to late appearance the threshold cycle. Melting curve analysis was performed post amplification to ensure the quality of PCR products as noted by the presence of a single peak. Briefly, the PCR plate was heated to 95^{o}C for 3 min, cooled to 55^{o}C then the temperature increased by 0.5^{o}C /sec up to 95^{o}C. Ovine glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**) was used as the reference gene and all gene expression levels were quantified and reported relative to GAPDH expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Real- time RT-PCR was performed in duplicate for each sample/primer set.

Statistical Analysis. One ewe (CN) was removed from the trial because it was discovered during surgery that she was carrying triplet fetuses. Data were analyzed in PROC MIXED of SAS 9.2 with gestational energy source and fetal number as fixed effects. Means were separated using LSD and considered significant when $P \leq 0.05$.

Results and Discussion

Mid- to late gestational energy source did not affect jejunal mRNA expression of *VEGF* ($P = 0.96$), *FLT1* ($P = 0.44$), *KDR* ($P = 0.36$), *NOS3* ($P = 0.53$), or *GUCY1B3* ($P = 0.73$) in the ewe (Table 3). Maternal small intestinal mass was also unaffected by gestational treatment in this study (Berg, 2012). Additionally, mRNA expression of these angiogenic factors was not affected ($P > 0.15$) by fetal number. Overall, ewe jejunal mucosal mRNA expression of *VEGF* and NO systems was not responsive to mid- to late gestational energy source, disproving our hypothesis.

In other ewe models of developmental programming, nutritional plane and Se supply during gestation have impacted jejunal mRNA expression of the *VEGF* and NO systems (Neville et al., 2010; Meyer et al., 2012b). Moreover, jejunal *KDR* and *NOS3* expression were correlated with intake in another study (Meyer et al., 2012a). Because DMI of ewes in the current study was greater for HL than CN and DG (2.03 vs. 1.17 and 1.18 kg/d; Berg, 2012), differences in angiogenic factor expression in the small intestine were expected. Unlike previous work in which nutrient intake and DMI were coupled by using varying levels of intake of the same basal

diet, nutrient intake did not follow DMI in the current study. Although fiber intake was greatest for HL, fat intake was greatest for DG, and protein intake was less for CN compared with both HL and DG. These differences in nutrient intake and the resulting differences in nutrient presence in the intestinal lumen may have lessened any possible alteration of gene expression due to DMI.

Although there was no effect of gestational energy source on fetal BW in the current study (Cretney et al., 2012), previous research using this model resulted in decreased birth weight of lambs born to ewes fed haylage compared with limit-fed corn or DDGS (Radunz et al., 2011a). Additionally, insulin sensitivity, post-weaning growth, and carcass characteristics were previously altered due to maternal energy source (Radunz et al., 2011b). Any differences between the current study and previous work that resulted in this observed lack of fetal growth response may also have affected the maternal small intestine.

In conclusion, VEGF and NO systems in the small intestine may not be involved in overall production responses to gestational energy source in ruminants. Based on this data, vascularity of the small intestine may not have played a role in observed dam and offspring responses in this model. Further research is planned to determine vascularity, growth indices, and nutrient transporter expression in these small intestinal tissues to determine if gestational energy source influences on the dam and fetus may be mediated at the maternal small intestinal level.

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Table 1. Ingredient and nutrient content of mid and late-gestation ewe diets

	Gestational Energy Source[1]		
Item	HL	CN	DG
Ingredient, %, DM basis			
Alfalfa haylage	100	12.1	12.2
Whole shelled corn	-	63.6	-
Corn dried distillers grains plus solubles	-	-	60.9
Supplement			
Ground corn	-	17.0	-
Corn dried distillers grains plus solubles	-	-	24.4
Soybean meal	-	4.85	-
Limestone	-	1.15	1.52
Monocalcium phosphate	-	0.12	-
Mineral & vitamin mixture[2]	-	0.97	0.97
Lasalocid[3]	-	0.02	0.02
Analyzed nutrient content, % DM			
CP	18.2	11.1	26.4
NDF	47.1	16.1	42.9
ADF	32.1	6.06	14.6
Ether extract	5.15	7.64	9.89
Ash	10.4	4.79	7.42
Ca[4]	1.10	0.81	0.89
P[4]	0.40	0.56	1.02
S	0.21	0.14	0.67

[1]HL = ad libitum fed alfalfa haylage and provided ad libitum access to a mineral and vitamin mixture (Sheep Mineral Plus,Vita Plus Corporation, Madison, WI); CN = limit-fed whole shell corn; DG = limit-fed corn dried distillers grains.
[2]Contained 56% NaCl, 9.5% Ca, 5.0% P, 1.5% Mg, 0.75% Zn, 0.40% Mn, 0.02% I, 0.005% Se, 0.0025% Co, 440,920 IU/kg vitamin A, 110,230 IU/kg vitamin D_3, 1,764 IU/kg vitamin E.
[3]Provided 27 mg of lasalocid (Alpharma, LLC, Bridgewater, NJ)/kg of dietary DM.
[4]The mineral and vitamin mixture was not included in the analyzed nutrient content of the HL diet.

Table 2. Sequence of primers used for ovine angiogenic factors and receptors

Gene of Interest	Description	Forward Primer	Reverse Primer
VEGF	Vascular endothelial growth factor	TGAGACCCTGGTGGACATCT	TATGTGCTGGCTTTGGTGAG
FLT1[1]	VEGF receptor 1	GTATCACTGCAAAGCCAGCA	AGCGTTAACAGGAGCCAGAA
KDR	VEGF receptor 2	AGCCGTTTGTTGCTTTCAGT	AGCACATGCCCACTTTAAC
NOS3	Endothelial nitric oxide (NO) synthase	GTGGAGATCAACCTGGCTGT	GACCATCTCCTGGTGGAAGA
GUCY1B3[1]	Soluble guanylate cyclase, binds NO	GAGGATGCCTCGCTACTGTC	CTGCTCCGTTTCCTCTGTTC

[1]Indicates that a bovine primer set was used.

Table 3. Effects of mid- to late gestational energy source on ewe jejunal angiogenic factor mRNA expression

	Gestational Energy Source				*P*-values	
Gene of Interest	CN	DG	HL	SEM	Energy Source	Fetal Number
VEGF	2.54	2.57	2.73	0.58	0.96	0.38
FLT1	0.075	0.798	1.048	0.545	0.44	0.72
KDR	1.9	31.6	42.6	20.0	0.36	0.81
NOS3	2.8	28.7	22.4	17.6	0.53	0.51
GUCY1B3	3.89	6.34	6.19	2.44	0.73	0.16

[1]CN =limit-fed whole shelled corn, DG = limit-fed corn dried distillers grains plus solubles, and HL = ad libitum alfalfa haylage.
[2]*FLT1* = *VEGF* receptor-1, *KDR* = *VEGF* receptor-2, *VEGF* = vascular endothelial growth factor, *GUCY1B3* = soluble guanylate cyclase, and *NOS3* = endothelial nitric oxide synthase 3.

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THE QUESTION REMAINS: "WILL THERE BE ENOUGH FOOD?"

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Introduction: We're living in exciting times! With a world population expected to reach 9 billion by the year 2050 and similar projections suggesting that our world's food supply needs will roughly double between now and then, students who are graduating from our land grant institutions in the next few years and launching their careers in animal agriculture will be in an enviable position. This time window of projected growth in food production will encompass much of their careers. Simply from the standpoint of supply and demand, it should be an economically rewarding time. There is definitely a need for the products our academic institutions produce: human resources and unbiased, research-based knowledge. However, we are operating in an entirely different environment than when the Land Grant System was initiated with the passing of the Morrill Act in 1862 which designated land in each state to be used for education in agriculture and the mechanical arts. The Morrill Act, supporting research and discovery, was followed by the Hatch Act in 1887. Finally, the Smith-Lever Act of 1914 established the third and final component of the Land Grant System, a network that extended information to producer and consumer clientele. Today's academic institutions exist in a time when the students they serve are accustomed to a world of instant information and instant gratification. How does that impact the Land Grant System and our mission in 2013? This presentation is intended to briefly review where we have been, assess where we are and to stimulate thought about what we need to do to satisfy world food production needs by 2050 and beyond.

The Challenge

One of the many seldom used sources of information on the over-flowing bookcases in my office is the USDA's 1981 Yearbook of Agriculture (1) entitled, "Will There Be Enough Food?" This was published a year prior to my arrival in Wyoming. The topic for the publication had been personally selected by John R. Block, an Illinois farmer who was the U.S. Secretary of Agriculture at that time. Having grown up on a small grain-livestock farm in east central Illinois in the 1950's and early '60's, there was always plenty of food. The question posed was not something that even crossed the mind of someone recently armed with a Ph.D. in the early 80's when I migrated west to Wyoming. I might point out to the bright minds in our current student body: one often finds wisdom in experience. Fast-forward another 30 years. Jerry Steiner, Executive Vice President of Sustainability and Corporate Affairs for The Monsanto Company, speaking at the Mendoza College of Business' "Ten Years Hence Speaker Series" at the University of Notre Dame in March of 2011 (Steiner, 2011), suggested that, with the world population expected to increase by a third by the year 2050 (to 9.2 billion people), food production needed to double. Multiple other organizations and agencies including the FASS-supported Farm Animal Integrated Research (FAIR 2012) report have echoed that same projection. I would once again pose the question that titles the 1981 Yearbook of Agriculture—"Will There Be Enough Food?" The production of a safe and wholesome food supply should be considered a national security issue.

Steiner went on to share some interesting statistics. He noted at that time that agriculture was using 66% of the world's fresh water and 55% of its farmable land. Since that time, with considerable marginal land brought into production because of the U.S. Energy policy and Renewable Fuels Act, that number has most probably increased. With more and more water and land being claimed by urban sprawl, much of the increased need for food production will have to come from developing technology. Steiner also shared additional insight into the impact of changing diets on the world food production system. In 1980, the average Chinese citizen consumed 32 pounds of meat per year. In 1995 it was 86 pounds and in 2007 consumption was 117 pounds, still only about half of what Americans eat. Improving economies typically drive these changes in diets. Based on World Bank economic data, Steiner noted that when people can afford to, they replace carbohydrates in their diet with protein provided by meat. Adding to the significance of the rapid increase in Chinese meat consumption is the fact that China currently has a population more than 4.28 times that of the U.S.

Importance of Animal Agriculture

The FAIR 2012 report cites USDA Farm and Ranch receipts for animal agriculture, including crops used as animal feeds, that currently total over $200 billion each year. They estimate that animal agriculture accounts for

between 60-70% of the total agriculture economy and plays an important role in agricultural trade. USDA projected that exports of livestock, poultry and dairy would reach $29.2 billion in FY 2012. The Food and Agriculture Organization (FAO) estimates that livestock contribute 40% of the global value of agriculture output and support the livelihood and food security of approximately 1 billion people.

FAIR 2012 cites research that shows that public investments in agricultural research yield a 20:1 return. However, federal funding for agricultural research has remained fairly stagnant at best over the past 20 years.

Future Research Needs

Three major themes or areas of importance emerged from the FAIR 2012 process. These were suggested as areas where investments in resources in animal sciences should be focused to meet future needs. Areas of importance included:

1. **Food Security** – The FAO projects that 70% of the needed doubling of food production will occur through the development and utilization of new technologies. They also estimated a 73% increase in meat consumption and a 58% increase in consumption of dairy products by 2050. Much of this increased consumption will be from countries with economies emerging from poverty that will demand enhanced diets.

   Food producers must embrace more sustainable practices, more efficiently utilize limited resources and make increased production efficiency a priority. These "waters are further muddied" by our government mandated emphasis on bioenergy which removes land and feed grains from food production systems and diverts them toward fuel production.

2. **One Health** – The interaction between animal and human health becomes more complex as globalization of animal agriculture continues to develop. Using an interdisciplinary approach, One Health addresses animal, human, and ecological health as well as their interactions.

   Policy and regulatory challenges are rampant in the in the intersection of animal and human health. Sound nutrition in both humans and livestock is a critical component of disease prevention and improved health. Animal health is also a key ingredient in animal well-being and efficient livestock production systems.

3. **Stewardship** – The utilization of natural resources in a sustainable manner provides balanced nutrition and fosters animal well-being. Food production systems must bear responsibilities for the environment and welfare of the animals within them.

Funding of Research

The recently formed National Association for the Advancement of Animal Science (NAAAS, 2013) states that overall public funding for agriculture research has been essentially stagnant for the past 30 years. Although livestock and the feeds they consume represented 60% of all agricultural sales receipts over the past 15 years, USDA directed 29% of their agricultural research funding to (CORRECT THE PHRASE THAT FOLLOWS:) animals and 71% to plants??? (1998-2010). This disparity may reflect the somewhat adversarial nature of livestock commodity groups when compared to plant commodity groups that tend to work in concert. Some researchers have been able to use livestock animal models to attract biomedical research dollars from NIH. In many cases this can be a win-win situation for both livestock production and human health. Whatever the reason, the simple fact remains that there is a lack of public funding for animal research which in turn puts more pressure on researchers to attract extramural funding. My concern is that young scientists who are working to generate adequate research productivity to achieve tenure often have to pursue non-agricultural extramural funding which may be at the expense of clientele or stakeholder needs. This dilemma is one that will remain a challenge to our land grant institutions as they strive to meet the needs of livestock producers in their state.

Shortly after becoming Department Head in 2001, the late Dr. Gordon Kearl, a retired Agricultural Economist from the University of Wyoming, presented me with a framed quote from Liberty Hyde Bailey, Dean of Agriculture and Experiment Station Director at Cornell University and dated 1907. It stated: "The University belongs to the people of the State. It will justify its existence only as it serves the people." I strongly agree with that statement. With the funding challenges confronting research programs, land grant institutions need to be reminded of Dean Bailey's quote.

The difficult economic times confronting research programs do foster institutional collaborations; for example, in just the past couple of weeks, animal scientists from Colorado

State University and the University of Wyoming worked alongside scientists from the University of Colorado Medical Center collecting tissue samples in our UW Meat Laboratory. These types of collaborative efforts are promoted by granting agencies and will lead to greater efficiencies in the use of ever-shrinking research funds.

Commodity groups will also need to step up to the plate to pick up some of the slack in research funding, especially with regard to research that is applied in nature. A considerable educational effort on the part of scientists and academic institutions will be required to nurture this partnership.

Graduate Education

Graduate students are the life blood and energy of any research program. We see students finely hone and develop their critical thinking skills in graduate school. They develop a deeper knowledge within their focus area of study which is generally narrower in scope than in undergraduate programs. Our student learning outcomes for our Master of Science students include possessing effective written and oral communications skills; they must also possess knowledge of scientific techniques for conducting and interpreting research as applied to animal agriculture; and, to possess skills to interpret and communicate science-based literature to agricultural consumers and producers.

Students earning a Doctor of Philosophy degree in Animal Science, in addition to the skills possessed by the M.S. student, will also possess skills to function as an independent researcher; possess skills in advanced research techniques; possess writing skills that will allow the development of grant proposals as well as peer-reviewed manuscripts for acceptance in the scientific literature and possess oral communication skills to facilitate presentation of data to science-based audiences. Overall, we want our students to exit their doctoral program equipped with skills that enable them to conduct innovative research, advancing scientific knowledge as well as making them competitive for positions as Principal Investigators.

I believe it is critical for graduate students to understand their research project from the most basic science at the molecular level to the implications of its impact at the applied, producer-level. When possible, we encourage our graduate students to have some interaction with commodity groups such as National Cattlemen's Beef Association, Wyoming Stock Growers' Association, American Sheep Industry, Wyoming Wool Growers Association and Wyoming and Colorado Meat Processors, to name a few. These interactions ensure that students understand and communicate the application of their research at the clientele level.

With shrinking federal and state funding, supporting graduate stipends will become more challenging with more of the burden being carried by extramural funding. We must continually ask ourselves, are we equipping students with the appropriate skills to be successful in this ever-changing environment?

Philosophy of Undergraduate Instruction

Undergraduate education may be under the most pressure from a culture that promotes instant information and gratification. Today's undergraduate students have information and communications at their finger-tips. I started to entitle this presentation "Remaining relevant in an Instant World." I believe maintaining relevance may be one of the greatest challenges facing our undergraduate programs. I would encourage instructors to utilize available technology in the classroom to keep students engaged. While sitting in on classes from time to time, I find about half of those bringing laptops, smart phones, iPads or other technological wizardry to class are using them appropriately to take notes or add to posted PowerPoint presentations (of course some are surfing the internet).

Several years ago we were asked to develop student learning outcomes for our major. In other words, if a student were to spend two, four or five years in our program, what besides grades and a degree would the student get for their tuition dollars. As a faculty, we decided that there were four outcomes critically important to student success. First, successful students are able to communicate. Written communications, oral communications and listening skills are all part of communication. A portion of the required communication courses are part of our University Studies Program or General Education requirements. However, we have also embedded writing and oral communications into several of our Animal and Food Science courses. Second, we believe successful students require a depth of knowledge within their discipline. We require students to earn a minimum grade of "C" in several of our core courses in Animal Science. This is not difficult for most students but such a requirement ensures certain depth of knowledge in the discipline. Third, I would contend that no matter what the profession, people deemed to be really good at what they do are those who can critically evaluate information and solve problems. That is just what life is about. A fourth component that I believe should receive more emphasis in our Student Learning Outcomes is ethics. I will address the issue of ethics as it relates to the entire Land Grant Mission at the end of this presentation.

Reduced budgets also make the funding of experiential learning opportunities such as meat, livestock, and horse judging opportunities difficult to maintain. Such activities contribute greatly to the student learning outcomes previously mentioned, especially critical thinking and communication skills. Furthermore, they enhance students' abilities to work together as a team. The opportunity to meet and network with industry leaders is another huge benefit to students as they leave their formal college training period.

The importance of internships cannot be overlooked. Since individuals have unique needs, we have not made internships mandatory for students but we strongly encourage them. I often tell students that internships not only allow them to apply what they have learned in the classroom to real life situations, but internships allow also them to transition from pursuing an education to pursuing a career. Increasingly, companies use a summer internship as a four-month job interview. At the end of the internship experience, if it has been a good experience for the employer and as well as for the student, the employer often suggests that they come back when they have completed their education and a job will be waiting for them. On the other hand, we have had students complete an internship and decide that this particular job is not something they want to do the rest of their life. They return to school and change their option or direction. It is important for students to find that out as well before they get out into the workforce. Internships are extremely valuable experiential learning experiences.

I believe some of our undergraduate courses would benefit from a blurring of Departmental lines. In other words, many of our course offerings could benefit from a more interdisciplinary approach. This is especially true of our production courses. Since I taught our Advanced Beef Production and Management course for several years, I'll use it as an example. We have proposed an Integrated Ranch Management Decision-Making Course that would be a two-semester course sequence covering ranch decision-making over the entire calendar year. It may well be team taught by instructors from Animal Science, Ag and Applied Economics, Range Management, Veterinary Science and Plant Science in a systems approach to problem-solving. I believe this approach is long overdue and should replace our traditional production courses. There may be some of our more traditional courses in nutrition that would include a plant science and/or Ag and Applied Economics component. Further, a reproduction course would have a Vet Science and/or economics component.

With more of our students coming from non-farm and non-ranch backgrounds, it is critically important that we continue to provide students with hands-on animal experiences. Laboratory opportunities providing animal handling experiences are critically important as we address animal welfare issues. The costs associated with Animal Science programs supplying these experiential learning experiences must remain within reason.

We are fortunate to be the only state-supported four year research institution in Wyoming. However, we have 7 community colleges in the state. We have done a pretty good job at the University-level of articulating courses and the course numbering system with our Wyoming Community College System. This makes transferring to our institution relatively seamless. In the past week it was announced that anyone graduating with an Associates of Science degree from a Wyoming Community College would be admitted to the University of Wyoming in a seamless fashion. However, we need to do a better job of articulating programmatic issues so students are more uniformly prepared in all areas of life sciences when they transfer to the four-year institution.

Clubs and organizations where students can develop leadership skills are also an important component of an undergraduate education. Such activities are often where students find their passion and become advocates for animal agriculture.

Undergraduate research programs are a fertile area for initiating a student's interest in graduate school. Our undergraduate research projects conclude with an Undergraduate Research Day where students write abstracts and present their research in either an oral or poster presentation. This is an avenue we need to expand in order to generate more interest in graduate programs, especially from our domestic undergraduate students.

Extension and Outreach Programs

In Wyoming, we have seen significant changes in delivery of extension educational programming over the past 30-plus years. When I first came to Wyoming as a Beef Cattle Extension Specialist in 1982, program delivery was largely by discipline. We see much more interdisciplinary programming using initiative teams and measuring impacts and performance in dollars and cents. This has been an extremely positive and effective approach to problem solving. Many granting agencies now require an outreach component which not only aids in the dissemination of the research-based information, but also engages Extension personnel in a deeper understanding of research procedures.

One of the most successful Extension Programs we have been involved in at the University of Wyoming has been the four-state Range Beef Cow Symposium that has been administered by the University of Wyoming, Colorado State University, the University of Nebraska and South Dakota State University for nearly 46 years. The program is held every other year and the site rotates among the four cooperating states. It has been a 2 ½ day program with the unique feature being Bull Pen Sessions during the evenings of the first two days. The speakers from their respective sessions during the day provide a panel for attendees, giving them an opportunity to ask their specific questions in the evening. This evening activity has been extremely successful and well-attended and is probably one of the main reasons the Symposium has survived for so long; attendance in recent years has ranged from about 650 to over 1000 producers and has included agribusiness personnel from as many as 18 states. The Symposium also includes a dynamic trade show numbering from 80 to approximately 100 exhibitors.

We have also seen effective, intensive hands-on 1 ½ day programs such as our WYO-Beef Short Courses that sometimes collaborates with industry or breed association sponsorship.

Extension education now uses a broad array of delivery methods and technology. In fact, Extension cannot afford to not use everything that is available in order to reach clientele. Webinars have become extremely effective and allow information to be extended over a wide geographical area and often received during convenient times for clientele. Websites are probably the most effective manner of reaching those who are technology savvy.

Of course, E-extension is growing and is considered by many to represent the future of educational program delivery. The challenge of this delivery system may be to be able to accommodate regional differences in environment.

Ethics

The University of Wyoming's College of Business and the Center for Cowboy Ethics and Leadership have developed a workshop using author James P. Owen's best-selling book "Cowboy Ethics" as its organizing concept. The initiative, **Standing Tall in an Upside-Down World**, was created with the goal of inspiring business executives to serve as principled leaders in their companies, industries, and communities at large. While originally created for a Wyoming audience, the program now impacts corporate and community leaders well beyond the state's borders.

The Standing Tall workshop was presented to our Beef Leadership class this past April. I was invited to attend the presentation and was so impressed that I am suggesting we expose our incoming freshmen to it and then provide a refresher session before they graduate. It is based on the following 10 principles under the Code of the West:

1. Live Each Day with Courage
2. Take Pride in Your Work
3. Always Finish What You Start
4. Do What Has to Be Done
5. Be Tough, But Fair
6. When You Make a Promise Keep It
7. Ride For the Brand
8. Talk Less and Say More
9. Remember that Some Things Aren't For Sale
10. Know Where to Draw the Line

A short video further illustrates these ten principles at the following link:

http://www.uwyo.edu/business/standing-tall/

Wyoming's state legislature passed a resolution signed by the Governor accepting these principles as the Wyoming State Code. We are the only state in the country to do so.

The People

As I look back on my career, my accomplishments owe much to the good fortune I had of being associated with some great people over all those years. It started at the University Illinois with the likes of Dr. A.L Neumann, friend and mentor, and Dr. D.E. "Gene" Becker, the Head of the Department of Animal Science at that time, who convinced me I had the ability to get a Ph.D, although the jury may still be out on that thought. Dr. Becker then convinced Dr. George Fahey and Dr. Darryl Kessler to take me on as a Ph.D. student. Dr. Frank Hinds who I had known at the University of Illinois and was Head of the Department of Animal Science at the University of Wyoming in 1981, recruited me to come to Wyoming where I've been for about 31 ½ years, the past 12-plus as Department Head. Over those years I've had the good fortune of working with an excellent faculty, staff and some fantastic graduate and undergraduate students. When you reach this point in a career, it is not what you have or have not done, but the people and friendships you have made that make the career special. I'm pleased to be able to count many of the more "chronologically enhanced" members of the WSASAS as my friends. For that, I thank you! I could go on for hours naming people who have had a significant impact on my life and career. The point being, however, is that we accomplish very little working alone. Throughout

each of our careers, we encounter people and groups of people who will have a synergistic effect on us as individuals in our professions. It will be those teams that move science forward to meet the challenges of the future.

Summary

When Dr. David Ames presented his Distinguished Service address four years ago (Ames, 2009), he stressed the importance of maintaining balance when "shift happens" in the academic environment. It is an exciting time as we strive to meet the demands of a growing global population that is projected to reach 9 billion people by 2050. Will the Land Grant System that was initiated more than 150 years ago meet the challenge with the assistance of the bright minds trained in the system over those years through the present? I wouldn't bet against them!

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