Proceedings

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2015-2016 WESTERN SECTION COMMITTEES

Executive

M. Salisbury, President - Angelo State University

S. Ivey, President-Elect - New Mexico State University

J. Berardinelli, Past President - Montana State University

C. Larson, Secretary-Treasurer - Zinpro Corporation, Eden Prairie, MN

K. Vonnahme, A & C Chair - North Dakota State University

J. Whittier, ASAS Director - University of Nebraska - Lincoln

B. Carter, Industry Director - Performiix

K. Quinn, Graduate Student Representative - New Mexico State University

H. Cunningham, Graduate Student Representative - University of Wyoming

Awards (3 year term)

‡, *J. Berardinelli - Montana State University (2015-16)

S. Lake - University of Wyoming (2013-16)

K. Vonnahme - North Dakota State University (2013-16)

R. Funston - University of Nebraska (2014-17)

G. Moss - University of Wyoming (2014-17)

C. Loest - New Mexico State University (2015 - 18)

S. Archibeque - Colorado State University (2015-18)

Beef Symposium (3 year term)

*E. Schollegerdes - New Mexico State University (2015-18)

A. Grove - AG Research LLC, White Sulphur Springs, MT (2013-16)

B. Neville - North Dakota State University (2013-16)

M. Ward - New Mexico State University (2014-17)

D. Bohnert - Oregon State University (2015-18)

C. Runyan - Angelo State University (2015-18)

R. Waterman - MT-USDA (2015-18)

Advising and Coordinating (3 year term)

*K. Vonnahme - North Dakota State University (2015-16)

‡S. Ivey - New Mexico State University(2015-16)

‡K. Quinn, New Mexico State University (2015-16)

H. Neiburgs, Washington State University (2014-17)

K. Cammack, University of Wyoming (2014-17)

J. Lamb, BYU-ID (2015-18)

S. Soto-Navarro - New Mexico State University (2015-18)

L. Prezotto - MT Research Station (2015-18)

Paper Competition (2 year term)

*C. Shauer, North Dakota State University (2015-16)

L. Hanna, North Dakota State University (2014-16)

J. Oltjen, University of California, Davis (2014-16)

D. Faulkner, University of Arizona (2014-16)

T. Hess, Colorado State University (2014-16)

A. Summers - New Mexico State University (2015-17)

J. Gifford - Oklamhoma State University (2015 - 17)

W. Stewart - Montana State University (2015-17)

S. Trojan - Texas Tech University (2015-17)

Academic Quadrathlon

*R. Endecott, - Montana State University

M. Kennedy, - Oregon State University

D. Kiesling, - University of Arizona

J. Lamb, - BYU-Idaho

K. DeAtley, - Chico State University

S. Archibeque, - Colorado State University

H. Han, - Colorado State University

S. Sato, - New Mexico State University

B. Bowman, - Utah State University

D. Rule, - University of Wyoming

Necrology

‡, *J. Berardinelli - Montana State University

Nominating

‡, **J. Berardinelli - Montana State University

‡, * J. Brett Taylor - USDA, ARS - Dubois, ID

‡G. Duff – Montana State University

ASAS Western Section Young Scholars Program

*R. Ashley ,New Mexico State University (2015-17)

M. Beckman, Zinpro Corporation (2016-018)

K. Dorton, Central Life Sciences (2016-018)

‡B. Carter, Industry Director (2014-16)

P. Hatfield, Montana State University (2014-16)

B. Alexander, University of Wyoming (2015-17)

R. Cook, Oregon State University (2015-17)

‡ K. Quinn, Graduate Student Representative, New Mexico State University (2015-16)

Undergraduate Poster Competition (2 year term)

*K. DeAtley, Chico State University (2016-17)

K. Cammack, University of Wyoming (2015-16)

C. Yeoman, Montana State University (2015-16)

C. Mueller, Oregon State University (2015-17)

T. Geary, USDA, ARS, Fort Keogh, Miles City, MT (2015-17)

Strategic Planning Committee

*C. Larson, Zinpro Corporation, Eden Prairie, MN

D. Hallford, New Mexico State University

J. Sprinkle, University of Arizona

R. Cooke, Oregon State University

K. DeAtley, Chico State University

A. Roberts, USDA/ARS Fort Keogh

S. Ivey, New Mexico State University

E. Sherman, Industry, private research facility

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* = Chair

‡ = Mandatory, not appointed

§ = Not appointed by WSASAS President

2015 WSASAS Annual Business Meeting

Ruidoso, NM

Prepared by Dr. Shanna Ivey, Secretary

Called to order: 8:35 MDT

Members Present: 37 members were present

Approval of Agenda: A motion to approve the agenda was put forth by John Hall/Bret Taylor. Motion passed

Approval of Minutes: Minutes were considered and a motion to approve the minutes was put forth by Bret Taylor/Tim Ross. No discussion, motion passed

REPORTS

Financial Report: Jacelyn Hemmelgarn and Shanna Ivey

WSASAS is maintaining at least 1 year's cost in our budget which is excellent. Jack Whittier commented that the proceedings seems to be costing more each year. It reflects the cost of the hard copy, difficulty collecting page charges. Mike Salisbury moved to accepted as presented, Joel Caton seconded. Pat Hatfield asked about registration for 2012 was it low because it was with JAM. Jacelyn confirmed that when we are with JAM we don't get all of our registration. Motion passed.

2015 Annual WSASAS meeting: Mike Salisbury

Registration: 211
Beef: 88
Sheep: 20
Extension: 62
Awards: 141
Grad Lunch and Learn: 50
Grad Mixer: 63
Horse Races: 83
Proceedings: 88 (92 submitted)

ASAS Report: Debra Aaron

Dr. Debra Aaron, ASAS president, provided a report of the activities of the national office.

Highlights:

- 6,200 members which is a record number of members. Increased comes from students
- Transitional membership for students to professional to maintain membership at a lower rate
- Services to members are increasing, ASAS resource guide is relaunching in August, Animal science database, 22K listings
- JAS is headed by Jim Sartin and Joel Caton has been very helpful. Impact factor 2.2
- ASAS and PSA sold their founding shares in FASS to ADSA. Ag guide was the concern when ASAS sold our third equity

interest in FASS. There is an animal care committee between ASAS, ADSA, PSA and a new guideline will be coming out and all three societies will work on the renewal. Updates are already in the works and the ag guide is taken care of. Public policy is the other piece of FASS, ASAS remains committed to public policy and it will be discussed at the board meeting in Florida.

- 2016 is the last JAM. ASAS will be meeting separately in 2017 and 2018. ADSA has been invited as our guest. We will still have dairy programming at ASAS.
- 2017 will be in Baltimore, 2018 in Vancouver

WSASAS President's Report: Jim Berardinelli
Recognized the executive committee and the ASAS national office. Jack Whittier asked that if anyone is interested in being the WSASAS representative to the National board…talk to Jack Whittier or Jim Berardinelli. Increased participation of extension specialists. We would like to do more programming with teaching. Beef and sheep Symposium committee was recognized for their efforts. Extension symposium organized Jim Sprinkle, Benton Glaze, Ken Olson was also a success.

WSASAS Committee Reports

AQ – Rachel Endecott

Advisors met during the contest and talked about what to do next year with JAM. For Salt Lake City the model used at the previous meeting would not work. They are proposing an April contest and Chico has agreed to host. There will also be tours and potentially talks about animal welfare. When the meeting was in Bozeman there were 9 teams and has been on the decline recently. Would like to take a critical look at having the AQ with the meetings due to challenges with student internship schedules, research responsibilities. Brenda Alexander asked that it is a challenge to get them here but don't they get some benefit. The problem is very few teams stay for the meetings due to costs, summer jobs, internships. Deb Aaron stated that all sections are facing the same challenges. Other sections have decided to keep it the same way. May need to open dialogue with the other sections to brainstorm solutions.

A and C – Connie Larson
Limited items for the committee to discuss. Clarify dates for 2017 NDSU meeting. Draft a travel policy nonmember speakers.

Awards –Bret Taylor
Nominations were submitted and evaluated by Wizehive and works well. Bohnert questioned that 3 only committee and Bret confirmed that they did divide the labor.

Beef Symposium – Reinaldo Cooke, Eric J. Scholljegerdes

Eric gave the report. 88 people. Enjoyed tours of NM and speakers at Corona.

Next year the beef symposium will support the Grazing Livestock Nutrition Conference. Jack Whittier asked Eric to expand on the dates and Ken Olson gave the report and the announcements usually held when JAM is in the west at a resort location. Will be in Park City, Utah July 18 and 19. Plans are underway for the program and details will be forthcoming.

Graduate Student Paper Competition – Brenda Alexander
There were 16 papers and the top 4 competitors were very close. 2 concerns by the committee – need a set policy on how to calculate the institutional award. In light of recent animal concerns need a requirement for all papers to have an IACUC statement. They would like to rewrite the guidelines to include this requirement.

Graduate Student Rep – Kelsey Quinn
55 attended the Lunch and Learn. Undergraduates are encouraged to attend. 5 student applied to be a candidate for grad representative.

Necrology – Bret Taylor
Dr. James Dennis Brinks retired from Colorado State University. Dr. G. Stan Smith retired from New Mexico State University. Dr. Paul Johnson Department Head at BYU (when they had an animal science program).

Nominating – Bret Taylor
Connie Larson was elected as secretary and will serve as the program chair for the WSASAS program with JAM in Salt Lake City, UT. Hannah Cunningham was elected new grad student director.

Undergraduate student poster competition – Kasey DeAtley
There were 9 posters representing 4 schools, NMSU, MSU, Chico, Oklahoma State University.

The committee with work on judging guidelines. Kasey would also like guidance on how it is going to work at JAM. Mike stated that there will be a designated time for our posters and plans are in the works. Other judges may need to be recruited. Dennis Hallford headed the efforts to fund the prize money. They are still seeking a more permanent funding source. Jim Sprinkle commented that the quality was excellent and the mentors and judges are to be commended for their efforts.

Young Scholars –Ryan Ashley, Shanna Ivey
Shanna gave the report. 2 qualified applicants. Awarded to Katelin Marchetti, NMSU for MS and Whit Stewart, NMSU, PhD

Old Business
Strategic planning report – Connie Larson
The survey can out 2x due to low number of respondents. Did not change the results dramatically.

New Business
Future site selection

- A letter and policy was drafted and there is a process to bid for future meeting sites. Site groups would put in bids discussed by the membership and voted on. This year we did solicitations and we have a number of universities

volunteer to host. Oregon State has volunteered to host in 2018 meeting in Oregon. Idaho has expressed interest as well for 2019. Wyoming has also been approached and she will ask her department head. Comments: Mike Salisbury asked if we should not just proceed for 2018 and decide today. Reinaldo Cooke stated that they would like to host in Bend, OR. Benton stated that they would try to host in Boise and would be in downtown with good access to food, activities and tours for beef symposium. Joel Caton/Rachel Endecott motion, motion passed. WSASAS 2018 will be in Bend, Oregon.

Streamlining abstract and proceedings forms

- Jacelyn Hemmelgarn stated that there are a huge number of forms to track all information needed for submission and lots of time is spent on this process. This is something that will be addressed and worked on during the year. Bohnert stated that as a session chair the process was cumbersome and difficult. Mike Salisbury stated that we are still in transition phase and hopefully the process will continue to improve and sort itself out.

Alternative formats for future annual meetings

- This came from strategic planning committee there seemed to be openness to pursuing alternative formats. There was some confusion on what this meant and a request was put out to clarify. Explore electronic formats.

Other business

- Plagiarizing issue with JAS and the proceedings. Reinaldo Cooke suggested that we change it enough so it will be less than 80%. We may need to add a footnote to indicate that this has been presented in part as a proceedings paper. The way the process works is that when a manuscript is submitted the authenticate software will flag regardless of the source. Boone Carter pointed out the proceedings is not a peer reviewed publication. Joel Caton stated that the proceedings is a launching point for a full manuscript and copyrights could be an issue.

Transfer of gavel
Jim Berardinelli to Mike Salisbury. Mike thanked Jim for his service and presented the past president plaque.

Meeting adjourned at 10:45.

REPORTS

Young Scholar Recognition Program Committee Report
Chair: Ryan Ashley

Committee Members:

J. Whittier	Colorado State University (2013-15)
G. Moss	University of Wyoming (2014-15)
‡B. Carter	Industry Director (2015-16)
P. Hatfield	Montana State University (2014-15)
B. Alexander	University of Wyoming (2015-16)
R. Cook	Oregon State University (2015-16)
‡ K. Quinn	Graduate Student Representative New Mexico State University

This year there were 2 Candidates representing 1 MS and 1 PhD (both from NMSU), which was a decline compared to previous year's nominees. The committee members that met at 2015 WSASAS propose that YSR nominees not be required to submit a proceedings for the nomination application starting next year. Additionally, the committee will be more proactive in encouraging colleagues to nominate students as well as encouraging graduate students to apply. Also, we suggest changing the terminology to include having the awardees present an "invited talk" as opposed to providing a "30 minute presentation". The members felt these measures will increase the number of applicants next year. The committee felt that the philosophy portion of the seminar that Whit included in his seminar was a very nice addition and suggest in the future we encourage other PhD winners to include a similar perspective. This YSR award is in its 3rd year and overall we feel it has been very successful and we look forward to next year's nominees.

2015 Young Scholar Recognition Recipients:
PhD Whit Stewart (NMSU) nominated by Dr. Eric Scholljegerdes
MS Katelin Marchetti (NMSU) nominated by Dr. Shanna Ivey

Strategic Planning Committee Report
Chair: Connie Larson

Committee members:
Dennis Hallford, NMSU
Shanna Ivey, NMSU
Jim Sprinkle, U of AZ
Reinaldo Cooke, OSU
Casey DeAtley, Chico
Andy Roberts, USDA Fort Keogh
Evin Sharman, Johnson Research, ID
Kelsey Quinn, NMSU

The committee sent out a membership survey to gather information on the current WSASAS mission and vision statements as well as the strategic plan. Respondent number was only 37 for the first time the survey was sent out. It was decided to send it out a second time and we had 43 respondents for a total of 80 respondents.

To summarize the demographics of the 80 respondents:

Membership
37 Professional members
3 Student members

43% more than 20 years
24% 11to 20 years
24% 6 to 10 years
10% 1 to 5 years.

Top ten areas of special interest:
13% Beef
12% Research
11% Ruminant Nutrition
8 % Applied Research
6% Teaching
6 % Physiology/Endocrinology
5% Extension
5% Growth/Development
5% Sheep/Goats
4% Industry

Top three job classifications
25% Teaching and Research
14% Research and Extension
11% Industry Technical Service

Over 91% of respondents agreed that the current vision and mission statements were still relevant. The a high majority of the responding membership felt that the WSASAS was fulfilling needs in terms of graduate student and professional development, dissemination of research, and viewed the Proceedings as a valuable source of research finding. A lower percentage of membership responded that WSASAS was fulfilling the needs for undergraduate student development, Extension programming and outreach, and teaching development.

Respondents strongly agreed that the four goals of the current Strategic Plan are still highly relevant:
1) Increase membership and participation
2) Increase relevance to members, producers and industry
3) Increase communication, and
4) Increase new initiatives.

While there is strong support for these goals, responses helped to provide direction for drafting a new Strategic Plan.

Two to three committee members are assigned to each Goal and will be working together to draft the new Strategic direction for respective goals. This is to be completed by the end of July. The full committee including the current and past Presidents will then review the full Strategic Plan and finalized a draft of the plan which will be forwarded to Kim Vonnahme, Chair of the Advising and Coordinating Committee for review and action by the Advising and Coordinating Committee.

Beef Symposium Report
Chair: Eric Scholljegerdes and Reinaldo Cooke

The 2015 Beef symposium was held from 7:30AM to 6:30PM on June 23. The Symposium consisted of two Ranch Tours in the morning and invited presentations in the afternoon.

During the morning session, the group toured two ranches. There first stop was the Greene Ranch, where Brian Greene talked about how range

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The presentations were:

- Understanding critical components of beef cow fertility and practical implementation to improve reproductive efficiency. G. Allen Bridges, Elanco Animal Health.
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- Sexed semen: Current commercial applications and what the future may hold for the beef producer John B. Hall, University of Idaho

The Symposium adjourned at 6:30PM

Advising & Coordinating Committee Report
Chair: Connie Larson (2015-2017)

Committee Members
M. Salisbury, Angelo State University (2013-15)
K. Quinn, New Mexico State University (2014-16)
J. Canton, North Dakota State University (2013-15)
T. Engle, Colorado State University (2013-15)
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1. Clarification of dates for the 2017 WSASAS Annual meeting to be held in Fargo, ND, June 20 to 23, 2017 (Tuesday through Friday).
2. Drafting the Travel Reimbursement Policy for Nonmember Invited Speakers.

2015 WSASAS Awards Committee Report
Chair: J. Bret Taylor

Committee Members:
J. Bret Taylor (Chair)
Margaret Benson
Terry Engle
Scott Lake
Kimberly Vonnahme
Rick Funston
Gary Moss

Call for award nominations was sent to the membership on January 12, 2015; deadline for nominations was March 20, 2015. Nominations by category were:

Distinguished Service: Drs. Daniel Rule and Michael Hubbert
Distinguished Teacher: Drs. Jason Ahola, Kristi Cammack, and Ryan Ashley
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Young Scientist: Drs. Reinaldo Cooke and Ryan Ashley

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Award winners by category were:
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Nominators of award winners were:
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Distinguished Teacher: Dr. Jack Whittier
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Young Scientist: Ms. Kelsey Quinn

Graduate Student Paper Competition
Chair: Brenda Alexander

Committee Members: Brenda M. Alexander (UW), Chair, Kris Johnson (WSU), Jennifer Thomson (MSU), Benton Glaze (UI), Lauren Hanna (NDSU), James Oltjen (UC, Davis), Dan Faulkner (UA), Tanja Hess (CSU), and Christopher Shauer (NDSU).

Six committee members were in attendance at the 2015 meeting including: B. Alexander, C. Johnson, B. Glaze, J. Oltjen, D. Faulkner, and C. Shauer. Additional written scores were submitted by L. Hanna and J. Thomson. Tanja Hess did not participate in any of committee's tasks.

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Proceedings and presentations were all high-quality. For scoring, an average score of the written (50 possible points) and oral presentation (50 possible points) was calculated and then combined for a total score (100 points). Six points separated the top four competitors. The top five placing were: 1) K. E. Quinn, NMSU; 2) B. I. Gomez, OSU (Oklahoma); 3) M.S. Crouse, NDSU; 4) J. L. Chase, OSU (Oklahoma); 5) K. C. Parkinson, USU.

Institutions were deemed eligible for the institutional award with two competitors. Institutions eligible for the 2015 award were: New Mexico State University (n=4), North Dakota State University (n=3), Oklahoma State University (n=2), Oregon State University (n=2). Colorado State

University, Montana State University, Texas Tech University, Utah State University and University of Wyoming were not eligible for the institutional award with only one competitor from each institution. For scoring, the top two scores from each institution were used to calculate an average institution score. Using this matrix Oklahoma State University was the top scoring institution with students placing 2nd and 4th. New Mexico State University placed a close second with their top students placing 1st and 7th.

Concerns regarding fairness and consistency for the institutional award were expressed by committee members. It was suggested, formal rules be adopted to calculate an institutional score. To make the process more transparent, adopted rules could be published along with the competition rules and guidelines.

In light of recent public concerns of animal welfare, it is the committee's recommendation that an explicit requirement for an animal use and care approval statement be instituted. Although this statement is required for Journal of Animal Science submissions and JAS style and format guidelines are followed for proceedings submission, an explicit requirement rather than an easily missed alluded to guideline would be helpful. It is the committee's opinion that this statement be required for acceptance to present. Of the 2015 submissions, two proceedings lacked any animal care and use approval statement, and IACUC approval was also not presented at the meeting. The concern is not that IACUC was not obtained; the concern is that this statement should be an expectation in all public presentations of research results.

Thank you for the opportunity to serve on this committee.

Undergraduate Poster Competition Committee Report
Chair: Kasey DeAtley

The second annual undergraduate poster competition was conducted on Wednesday, June 24, 2015 at the Ruidoso Convention Center, Ruidoso, NM. A total of nine undergraduates submitted abstracts for the poster session. Institutions represented included Oklahoma State University, Montana State University, New Mexico State University, and California State University, Chico. Committee members that served as judges included Dr. Tom Geary, Dr. Kristi Cammack, and Dr. Kasey DeAtley (chair). Winners of the poster session were:

1st Place: J. A. Matera, Oklahoma State University
2nd Place: A.L. Garza, New Mexico State University
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Monetary awards included $250 for 1st Place, $150 for 2nd Place, and $100 for 3rd place. Committee members are seeking additional sponsors for the 2016 contest and will be editing poster score sheet and procedures.

APPENDIX 1

2015 WSASAS BUSINESS MEETING AGENDA

Ruidoso, NM Convention Center
Aspen Room
Friday, June 26, 2015

Call to Order – Jim Berardinelli

Approval of the Agenda – Jim Berardinelli

Approval of the 2014 WSASAS Business Meeting minutes – Shanna Ivey

Reports

- 2015 Financial – Jacelyn Hemmelgarn, Shanna Ivey
- 2015 Annual WSASAS Meeting – Mike Salisbury
- ASAS President – Debra Aaron
- WSASAS President – Jim Berardinelli
- WSASAS Committees
  - Academic Quadrathlon – Rachel Endecott
  - Advising and Coordinating – Connie Larson
  - Awards – Bret Taylor
  - Beef Symposium – Reinaldo Cooke, Eric Scholljegerdes
  - Graduate Student Paper Competition – Brenda Alexander
  - Graduate Student Representative Report – Kelsey Quinn
  - Necrology – Bret Taylor
  - Nominating – Bret Taylor
  - Undergraduate Student Poster Competition – Kasey DeAtley
  - Young Scholars Program – Ryan Ashley

Old Business

- Strategic Planning Survey: Results and Actions

New Business

- Future site selection: will the new policy work for WSASAS
- Streamlining Abstract and Proceedings forms
- Alternative formats for future Annual Meetings

Transfer of the Gavel

Adjourn

APPENDIX 2

AMERICAN SOCIETY OF ANIMAL SCIENCES
STATEMENT OF ACTIVITIES
WESTERN SECTION

	YTD 12/31/14	YTD 12/31/13	YTD 12/31/12	YTD 12/31/11
Revenue and Support				
Ticketed Events	7,500	9,875	3,360	4,139
Donations and Sponsorships	5,500	5,625	1,425	1,250
Proceedings	4,365	8,385	10,745	9,090
Registrations	13,965	20,146	1,566	20,551
Investment Earnings Gain (Loss)	1,989	6,922	5,246	(873)
Total Revenue and Support	33,319	50,953	22,342	34,157
Expenses				
Programs/ Registration	3,356	1,297	2,292	221
Awards/Plaques	13,935	13,898	6,235	5,950
Convention Center	6,982	17,125	1,716	10,371
Marketing	3,841	3,302	865	3,273
Proceedings	3,036	4,021	2,348	1,407
Postage, Shipping & Supplies	1,329	363	17	984
Miscellaneous	7,186	7,183	2,707	7,895
Insurance	-	-	195	111
Staff Support	(1,253)	6,553	3,784	10,802
Total Expenses	38,412	53,742	20,159	41,014
Change in Net Assets	(5,093)	(2,789)	2,183	(6,857)
Net Assets, Beginning of Period	50,943	53,732	51,549	58,405
Net Assets, End of Period	$ 45,850	$ 50,943	$ 53,732	$ 51,548

APPENDIX 3

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APPENDIX 8

Young Scholar Recognition Program Committee Report
Chair: Ryan Ashley

Committee Members:

J. Whittier	Colorado State University	(2013-15)
G. Moss	University of Wyoming	(2014-15)
‡B. Carter	Industry Director	(2015-16)
P. Hatfield	Montana State University	(2014-15)
B. Alexander	University of Wyoming	(2015-16)
R. Cook	Oregon State University	(2015-16)
‡ K. Quinn	Graduate Student Representative New Mexico State University	

This year there were 2 Candidates representing 1 MS and 1 PhD (both from NMSU), which was a decline compared to previous year's nominees. The committee members that met at 2015 WSASAS propose that YSR nominees not be required to submit a proceedings for the nomination application starting next year. Additionally, the committee will be more proactive in encouraging colleagues to nominate students as well as encouraging graduate students to apply. Also, we suggest changing the terminology to include having the awardees present an "invited talk" as opposed to providing a "30 minute presentation". The members felt these measures will increase the number of applicants next year. The committee felt that the philosophy portion of the seminar that Whit included in his seminar was a very nice addition and suggest in the future we encourage other PhD winners to include a similar perspective. This YSR award is in its 3rd year and overall we feel it has been very successful and we look forward to next year's nominees.

2015 Young Scholar Recognition Recipients:
PhD Whit Stewart (NMSU) nominated by Dr. Eric Scholljegerdes
MS Katelin Marchetti (NMSU) nominated by Dr. Shanna Ivey

APPENDIX 9

Strategic Planning Committee Report
Chair: Connie Larson

Committee members:
Dennis Hallford, NMSU
Shanna Ivey, NMSU
Jim Sprinkle, U of AZ
Reinaldo Cooke, OSU
Casey DeAtley, Chico
Andy Roberts, USDA Fort Keogh
Evin Sharman, Johnson Research, ID
Kelsey Quinn, NMSU

The committee sent out a membership survey to gather information on the current WSASAS mission and vision statements as well as the strategic plan. Respondent number was only 37 for the first time the survey was sent out. It was decided to send it out a second time and we had 43 respondents for a total of 80 respondents.

To summarize the demographics of the 80 respondents:

Membership
37 Professional members
3 Student members

43% more than 20 years
24% 11to 20 years
24% 6 to 10 years
10% 1 to 5 years.

Top ten areas of special interest:
13% Beef
12% Research
11% Ruminant Nutrition
8 % Applied Research
6% Teaching
6 % Physiology/Endocrinology
5% Extension
5% Growth/Development
5% Sheep/Goats
4% Industry

Top three job classifications

25% Teaching and Research
14% Research and Extension
11% Industry Technical Service

Over 91% of respondents agreed that the current vision and mission statements were still relevant. The a high majority of the responding membership felt that the WSASAS was fulfilling needs in terms of graduate student and professional development, dissemination of research, and viewed the Proceedings as a valuable source of research finding. A lower percentage of membership responded that WSASAS was fulfilling the needs for undergraduate student development, Extension programming and outreach, and teaching development.

Respondents strongly agreed that the four goals of the current Strategic Plan are still highly relevant:

1) Increase membership and participation
2) Increase relevance to members, producers and industry
3) Increase communication, and
4) Increase new initiatives.

While there is strong support for these goals, responses helped to provide direction for drafting a new Strategic Plan.

Two to three committee members are assigned to each Goal and will be working together to draft the new Strategic direction for respective goals. This is to be completed by the end of July. The full committee including the current and past Presidents will then review the full Strategic Plan and finalized a draft of the plan which will be forwarded to Kim Vonnahme, Chair of the Advising and Coordinating Committee for review and action by the Advising and Coordinating Committee.

ANIMAL BEHAVIOR AND WELL-BEING

Proceedings, Western Section, American Society of Animal Science

Vol. 67, 2016

Grazing behavior and production characteristics among cows differing in residual feed intake while grazing late season Idaho rangeland[1]

J. E. Sprinkle*,†,[2], J. B. Taylor‡, P. E. Clark§, M. C. Roberts-Lew†, and J. B. Hall*,†
*Department of Animal and Veterinary Sciences, University of Idaho, Moscow 83844
†University of Idaho Nancy M. Cummings Research, Extension & Education Center, Carmen 83462;
‡USDA Agricultural Research Service, U. S. Sheep Experiment Station, Dubois 83423
§USDA Agricultural Research Service, Northwest Watershed Management Research Unit, Boise 83712

ABSTRACT: The objectives were to determine if cows classified as either low- or high-residual feed intake **(LRFI** or **HRFI**) differed in BW, BCS, and winter grazing activity over time. Thirty Hereford x Angus (LRFI = 16; HRFI = 14) 2-year-old cows grazed sagebrush-steppe for 78 d beginning 29 September 2016. Body weight and BCS were collected before and after grazing. Five cows of each RFI classification were fitted with global-positioning-system (**GPS**) collars on 16 November 2015 with data collection commencing 3 d later and continuing for 25 d in a 323-ha pasture. The GPS units collected location coordinates every 2 min from which total daily travel was calculated. Visual counts for bite rate were obtained from collared cows over 8 d. Coordinate data, daily bite rate, BW, and BCS were analyzed as repeated measures using a mixed model, which included RFI group, day, and RFI group x day as fixed effects and cow within RFI group as the random effect. Change in BW and BCS were analyzed by ANOVA with RFI group as the main effect. Cow BCS and BW differed for both day ($P < 0.0001$) and day x RFI ($P < 0.05$). Body condition was less in LRFI cows at the beginning (5.8 ± 0.13 vs 6.2 ± 0.14 BCS), but similar to HRFI at the end of the study (4.6 ± 0.13 vs 4.6 ± 0.14). Body weight for the different RFI cows did not differ ($P = 0.1974$) prior to going to range. However, BW-change and BCS-change differed ($P = 0.05$) among RFI groups. Not only did the LRFI cows lose less BW (-50.0 ± 5.41 kg vs -66.6 ± 5.78 kg) over the trial, they also were less variable with respect to BW loss. Cows did not differ ($P > 0.21$) by RFI for distance travelled or bite rate, but day was significant ($P < 0.0001$) with cows increasing bite rate as the season of year progressed (55.2 ± 5.63 bites/min for d 4 vs 84.8 ± 5.32 bites/min for d 21) and increasing distance travelled as snow storms occurred. Although LRFI cows were leaner than HRFI cows at the commencement of the project, they loss less BW and functioned competitively in a late season rangeland environment.

Key Words: Beef Cattle, Grazing behavior, Rangeland, Residual feed intake

Introduction

Residual feed intake (**RFI**) is expressed as the difference between expected feed intake (based upon body weight and growth) and actual feed intake (Koch et al., 1963). Although intensive research and industry adoption (Wulfhorst et al., 2010) of selection for RFI has been practiced over the last two decades, limited research has been conducted with divergently selected RFI cattle in a challenging rangeland environment (Knight et al., 2015). Biological, mechanistic drivers of cow performance may differ by age, stage of production, and availability of feed resources. For example, Randel and Welsh (2013) stated that selection for low RFI resulted in selection of heifers that are leaner, reach puberty at older ages, and calve later in their first and subsequent calving seasons. Yet, when evaluating cows over 10 production cycles which produced low-RFI (**LRFI**) and high-RFI (**HRFI**) progeny, Basarab et al. (2007) found that cows which produced LRFI (more efficient) progeny had 2 to 3 mm more backfat than dams that produced HRFI (less efficient) progeny. In a separate study, a decline in fertility was reported for low RFI heifers in a grazing environment (Basarab et al., 2011). It appears there may be a threshold for decreased body energy stores that exists among younger LRFI heifers which can be overcome as they mature and are able to express an advantage with maintenance requirements in a grazing environment. It is important to determine "fitness" of young 2-yr-old cows differing in feed efficiency when presented to a challenging rangeland environment.

In an Idaho sagebrush-steppe environment, the objectives were to determine if cows classified as either LRFI or HRFI differed in BW, BCS, and grazing behavior (bite rate and distance travelled) over time.

Materials and Methods

Range site. The main study site for this experiment was in a 323-ha pasture on the USDA, ARS, U. S. Sheep Experiment Station (**USSES),** located about 16 km northeast of Dubois, Idaho (112° 7' W 44° 18' N). Water troughs were located centrally in the pasture and refilled on a daily basis, usually around noon. This range site is in the sagebrush-steppe with elevations within the pasture ranging from 1762 to 1806 m on slopes less than 20% but mostly between 0 to 12 percent. The mean annual precipitation (1981 to 2010) near the research site (112° 12' W 44° 15'N, elevation, 1661 m) is 328 mm with 58% falling during April through September. The pasture is dominated by mountain big

[1]We acknowledge the support of the Idaho Experiment Station and USDA ARS employees N. Strong for GPS data management and processing; R. Laird, B. Leonard, and J. Hensley for animal care and management; and M. Williams for project coordination.
[2]Corresponding author's e-mail: sprinkle@uidaho.edu

sagebrush (*Artemisa tridentate* Nutt. *ssp. vaseyana* [Rydb.] Beetle) and threetip sagebrush (*A. tripartiae* Rydb.) with subdominant shrub species including antelope bitterbrush (*Purshia tridentata* [Pursh] DC.), yellow rabbitbrush (*Chrysothamnus viscidiflorus* [Hook.] Nutt.), and spineless horsebrush (*Tetradymia canescens* DC.). Dominant perennial grasses include thickspike wheatgrass (*Elymus lanceolatus* [Scribn. & J.G. Sm.] Gould ssp. lanceolatus), bluebunch wheatgrass (*Pseudoroegneria Spicata* [Pursh] A. Löve ssp. spicata), and plains reedgrass (*Calamagrostis montanensis* Scribn. ex Vasey) with only trace amounts of annual cheatgrass (*Bromus tectorum*). The domant forb on this site is silvery lupine (*Lupinus argenteus* Pursh). Soils are mapped as complexes of Maremma (Fine-loamy, mixed, superactive, frigid Calcic Pachic Argixerolls), Pyrenees (Loamy-skeletal, mixed, superactive, frigid Typic Calcixerolls), and Akbash (Fine-loamy, mixed, superactive, frigid Calcic Pachic Argixerolls) soils.

Forage Production. Forage production was estimated at the beginning of the grazing period by hand clipping 10 randomized 0.16 m^2 quadrats from both a site with a burn history and one without a burn history from within this sagebrush-steppe. All perennial and annual graminoids rooted within the quadrat frame within the two sampled areas were clipped to ground level and dried for 24 h at 65°C. Sagebrush canopy was not sampled for production.

Animals and Grazing Behavior. All procedures were approved by the University of Idaho Animal Care and Use Committee (IACUC # 2015-44). Previous to this trial, all of the predominantly Hereford x Angus 2-yr-old cows used in this study were classified for RFI as yearling heifers following the protocol described by Hall et al. (2015). Heifers in that study were classified to be either average, inefficient (HRFI), or efficient (LRFI) with scores categorized by their standard deviation according to the contemporary mean. Due to our desire to compare young cows for rangeland adaptability who varied greatly in feed efficiency, only 2-yr-old HRFI and LRFI cows were used, with 14 HRFI and 16 LRFI cows being utilized.

Preceding and following data collection on rangeland, all cattle were weighed and scored for BCS (1 to 9, 9 = fattest) on 21 September and 16 December 2015. On 29 September 2015, 20 d after weaning of calves, cattle were shipped with the cowherd (n =242) from the Nancy M. Cummings Research, Extension and Education Center located at Carmen, Idaho to the USSES Henninger range near Kilgore, Idaho. Cattle grazed at Henninger for 46 d and were then trailed to the USSES Headquarters range over a 2-d period. Prior to trailing, 5 of the HRFI and 5 of the LRFI cows were instrumented with custom global-positioning-system (**GPS**) collars (Clark et al., 2006) programmed to obtain GPS positions every 2 min from which daily travel distances (**DTD**) were calculated over the 27-d trial.

The collared cattle accompanied the rest of the experimental cattle throughout the remainder of the grazing period.

Eight days of grazing observations were conducted on horseback by one observer for 3 to 4 h in morning (about 0730 to 1130 local) and afternoon (1300 to 1700). The same observer and horse was used on all days of the study. Observational data were collected on d 4, 6, 7, 14, 15, 16, 20, and 21 of the trial corresponding to 21, 23, and 24 November and 1, 2, 3, 7, and 8 December 2015. Cattle were able to be approached closely by the horseback observer (to within 6 m) and did not exhibit flight response or agitation during the observation process. On some days (n = 4), one or two cows were unable to be found but on other days (n = 3), a majority of the cattle were observed twice (i.e. AM and PM).

Focal sampling for bite rate (bites/min) was conducted on single animals (Sprinkle et al., 2000) during the AM and PM observation time periods. At least 4 replicate samples per observation period were acquired whenever possible. Beginning and ending times for each replicate were recorded in the field on a tablet computer using a spreadsheet with an integrated timestamp. Sometimes cattle commenced resting, walking to water, or ruminating in the midst of an observed grazing bout, so it was not always possible to obtain multiple sample replicates of 4 or greater within each grazing observation period. Bite rate frequency data were averaged over each AM or PM time period and then daily bite rate was averaged over both AM and PM grazing.

Grazing Collar Data Management. The GPS data from the 2-d trailing event and the first and last days of grazing were omitted due to the confounding effects of herding on cattle behavior. The GPS positions appearing > 5 meters outside of the mapped fenceline were treated as outliers and deleted. Positions ≤ 5 m outside of the fence were kept since the location outside the fence was most likely due to a combination of minor GPS and map errors. To help identify the paths traveled, lines were generated from point to point and split by date. After visually inspecting all points with positional-dilution-of-precision (**PDOP**) values > 6.0 for the first collar processed, it was determined that all points with a PDOP ≥ 10.0 could be automatically deleted from the remaining data sets before conducting further review. All remaining points with a PDOP > 6.0 were individually inspected for fit with the surrounding points. Flagged positions fitted along the travel trajectory line sharply diverging from the general path were deleted. In some instances where several consecutive points had similar high PDOP values, the number of satellites used for each point was also used to determine which positions to keep or discard. Ideally, each GPS location should have had at least 5 satellites present, but many were based on only 3 or 4 satellites. Several points with PDOP values between 6.0 and 7.0 were kept because of the higher number of satellites used and the good fit with the surrounding points. Additional points with PDOP values 6.1 to 7.0 and a few around 8.0 and 9.0 were kept to maintain the general shape of the path traveled in instances where several consecutive points had PDOP values > 6.0.

Obvious point outliers due to GPS error were deleted. For example, points sharply diverging from the trajectory path by ≥ 20 m, while an animal appeared to be stationary, were deleted. Furthermore, points in line with surrounding points, but having a high PDOP value, were either deleted due to its poorly-rated quality and low influence on the overall path, or kept to maintain the overall shape of the path if it was part of a group of points with high PDOP values. Also, some points with PDOP of < 6.0 were deleted if points were over 100 m from a cluster of other points collected from an apparent resting cow. For 9 of the 10 collars used, < 10%

of the 17,000-plus data points were deleted.

After deletion of erroneous GPS positions, a new set of directional path lines were generated for each cow and examined for any additional outliers to be deleted. From the final corrected path line, DTD for each cow was calculated.

Statistical Analyses. Daily bite rate, DTD, and production (cow BW and BCS) data were analyzed using a restricted maximum likelihood-based mixed effects model for repeated measures (v. 9.4, SAS Inst., Inc., Cary, NC) with the categorical, fixed effects of RFI group and day of study (or day of year for repeated production data) and the interaction between RFI group x day of study (or year). Cow within RFI group was included as a random effect and was the repeated subject. The denominator degrees of freedom for treatment *F*-statistics were approximated using the Kenward-Roger's method. For all these models except DTD, a heterogeneous autoregressive structure was used as a covariance structure to model the relationships between repeated observations. In order to get distance travelled to properly converge, a simplified compound symmetry covariance structure was used for this model. The change in BW and change in BCS had no repeated data and were analyzed using the GLM procedure of SAS (v. 9.4, SAS Inst., Inc., Cary, NC) with RFI group as a main effect. Least squares treatment means for all statistical models were separated using the pairwise contrasts (PDIFF, v. 9.4, SAS Inst., Inc., Cary, NC). Letter assignments for differences in least square means were produced using the pdmix800.sas macro as originally described by Saxton (1998).

Results and Discussion

Forage Production. Forage production for perennial and annual grasses combined was estimated at 331 ± 95 kg/ha for the unburned part of the pasture and 491 ± 242 kg/ha for the burned part. Approximately 75% of the pasture was burned in 2005, so the weighted-estimated-average forage production on the entire pasture was 451 ± 205 kg/ha. Assuming maximal average forage intake was 2.1% of BW for the entire 242 head of cattle in this pasture, then DMI was estimated to be 12.14 kg/cow for cows averaging 578 kg BW at a BCS = 5. Over 26 d (cows fed hay on the first day they arrived in the pasture), forage intake was estimated to be approximately 76,385 kg which was approximately 52% of the available forage (145,673 kg) on display. At this level of forage utilization, cattle would not be forced to do extensive searching to obtain sufficient daily forage. Based on animal performance (Table 1), cattle may have consumed less forage than the maximal amount described.

Animal Performance. In agreement with published literature (Kerley, 2010), the 2-yr-old LRFI cows in this study were leaner ($P = 0.0196$) prior to going to rangeland. By the end of the grazing period, BCS did not differ ($P = 0.6673$) among the two groups (Table 1), presumably due to lower daily maintenance requirements for the LRFI group. Simple means (Hall et al., 2015) for the yearling heifer RFI scores in units of standard deviation were 1.17 ± 0.2 for HRFI and -0.88 ± 0.1 for the LRFI heifers. Means for BCS were significant for the interaction of day of year with RFI classification ($P = 0.0309$). Day of year was significant at ($P < 0.0001$).

As with BCS, BW was significant for the day of year effect ($P < 0.0001$) and the interaction ($P = 0.0483$) of RFI group with day of year (Table 1). In contrast to the results for BCS, BW for the different RFI cows did not differ ($P = 0.1974$) prior to going to range (Table 1). However, BW change and change in BCS differed significantly ($P = 0.0456$ for BW and $P = 0.0296$ for BCS change) among the LRFI and HRFI cows in this study following 78 d of grazing late season Idaho rangeland (Table 1). Not only did the LRFI cows lose less BW over the trial, they also were less variable with respect to BW loss (Fig. 1), indicating more opportunity for making positive changes in overall feed efficiency in the cow herd by careful selection among selected tested sires.

Grazing Behavior. A central concern for young efficient cattle is whether or not these leaner animals will effectively function in a more limiting rangeland environment when compared to inefficient animals with more aggressive feed intake and greater body condition (at least as younger animals). We found no differences (Table 2) between the LRFI and HRFI groups for either DTD ($P = 0.5320$) or bite rate ($P = 0.2158$). Similarly, the interaction of RFI group with the day of the study was not significant for DTD ($P = 0.8262$) or bite rate ($P = 0.5915$). However, the influence of the day of the study was highly significant ($P < 0.0001$) for both behavioral responses.

As the season of year progressed, bite rate increased for all cows ($P < 0.05$; Table 2). We interpret this to be a behavioral response to promoted increased harvesting efficiency as temperatures decreased and daylight hours declined.

Significant increases ($P < 0.05$) in DTD were associated with snow storms which occurred on 25 November, 30 November, and 13 December 2015 (Table 2). It is apparent by reviewing these data that the cows in this study changed behavior as a result of the winter storms, likely due to seeking shelter and being driven by the wind (Valentine, 2000; Rubio et al., 2008). Substantial changes in travel distance were evident when data for the day prior to the storm event were contrasted with that from the day of the storm. For example, when a big snow storm moved in on December 13, 2015, travel increased ($P < 0.0001$) from 8.2 ± 0.42 km/d the day before the storm to 14.3 ± 0.42 km/d on the day of the storm.

Very little research has been conducted comparing cows differing in RFI on rangeland. Knight et al. (2015) evaluated grazing behavior for low RFI (efficient) vs high RFI (inefficient) cows on Arizona rangeland. Efficient cows travelled further ($P = 0.005$) than inefficient cows (6.23 km/d vs. 5.84 km/d) on pinyon-juniper rangelands but there was no difference ($P \geq 0.06$) for distance travelled, distance from water, and elevation for Ponderosa pine rangeland. Additional research data is needed to better describe conditions that drive and determine foraging efficiency on rangeland in different environments. In our study, we did not see any differences in distance travelled on late season rangeland in Idaho with non-lactating 2-yr-old cows differing in RFI.

Implications

The young, 2-yr-old LRFI cows in our study were leaner than HRFI cows prior to being moved to rangeland. However, the LRFI cows lost less weight and BCS than RFI cows while wintering on rangeland. Furthermore, LRFI cows appear to have less variability for weight loss than do HRFI cows, leading one to conclude there may be opportunity for selection of efficient cows that eat less and also fit a rangeland environment. However, further studies need to be done with lactating 2-yr-old cows on rangeland to see if these leaner cows can survive in a rangeland environment.

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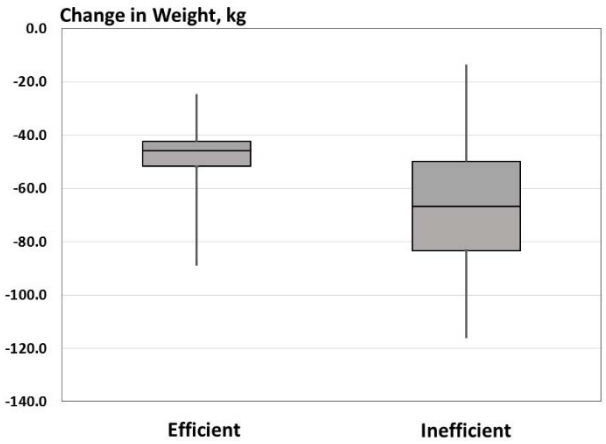

Figure 1. Box plot of distribution of weight loss data for efficient (Low RFI) vs inefficient (High RFI) 2-yr-old cows grazing late season Idaho rangeland for 78 d. Center line in each box is median value, highest and lowest point of whiskers indicate maximum and minimum values. Upper section of box is the first quartile of data and lower section is the third quartile of data.

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Table 1. Performance data for cows differing in RFI[1]

Item	LRFI cows[2]	HRFI cows[2]	*P*-value
Wt, kg			
Beginning	556.8 ± 10.1	576.8 ± 10.8	0.1974
Ending	506.8 ± 9.8	509.8 ± 10.5	0.8363
BCS			
Beginning	5.8 ± 0.13[a]	6.2 ± 0.14[b]	0.0196
Ending	4.6 ± 0.13	4.6 ± 0.14	0.6673
Wt change, kg	-50.0 ± 5.41[a]	-66.6 ± 5.78[b]	0.0456
BCS change	-1.2 ± 0.11[a]	-1.6 ± 0.12[b]	0.0296

[1]Cows were on rangeland for 78 d, RFI = residual feed intake
[2]LRFI = 16 low RFI efficient cows; HRFI = 14 high RFI inefficient cows
[a,b]Means within row with different superscripts differ, ($P < 0.05$).

Table 2. Grazing behavior for two-year-old cows differing in feed efficiency.

Date	Day of study	*n*	**Distance travelled, km/d**	SEM	*P*-value
Distance travelled by day					
19-Nov-2015	2	10	4.21[l]	0.422	< 0.0001
20-Nov-2015	3	10	4.89[kl]		
21-Nov-2015	4	10	6.57[fghi]		
22-Nov-2015	5	10	6.04[hij]		
23-Nov-2015	6	10	6.99[efgh]		
24-Nov-2015	7	10	6.00[hij]		
25-Nov-2015	8	10	11.32[b]		
26-Nov-2015	9	10	11.47[b]		
27-Nov-2015	10	10	11.27[b]		
28-Nov-2015	11	10	7.81[de]		
29-Nov-2015	12	10	6.40[fghij]		
30-Nov-2015	13	10	9.01[c]		
1-Dec-2015	14	10	6.02[hij]		
2-Dec-2015	15	10	8.32[cd]		
3-Dec-2015	16	10	6.40[fghij]		
4-Dec-2015	17	10	5.53[jk]		
5-Dec-2015	18	10	5.70[ijk]		
6-Dec-2015	19	10	7.09[efg]		
7-Dec-2015	20	10	6.28[ghij]		
8-Dec-2015	21	10	4.88[kl]		
9-Dec-2015	22	10	7.02[efgh]		
10-Dec-2015	23	10	8.13[cd]		
11-Dec-2015	24	10	7.33[def]		
12-Dec-2015	25	10	8.24[cd]		
13-Dec-2015	26	10	14.35[a]		
Distance travelled by treatment group					
Efficient (LRFI)[1]	All d	5	7.34[a]	0.305	0.5320
Inefficient (HRFI)[1]	All d	5	7.63[a]		
Date	Day of study	*n*	**Bites/min**		*P*-value
Bite rate by day of study					
21-Nov-2015	4	9	55.2 ± 5.63[e]		< 0.0001
23-Nov-2015	6	9	66.3 ± 6.21[ab]		
24-Nov-2015	7	7	74.3 ± 5.49[ac]		
1-Dec-2015	14	10	64.2 ± 5.63[b]		
2-Dec-2015	15	9	65.6 ± 5.35[b]		
3-Dec-2015	16	10	81.3 ± 5.24[cd]		
7-Dec-2015	20	10	82.9 ± 5.14[d]		
8-Dec-2015	21	10	84.8 ± 5.32[d]		
Bite rate by treatment group					
Efficient (LRFI)[1]	All d	5	78.4 ± 3.72[a]		0.2158
Inefficient (HRFI)[1]	All d	5	65.3 ± 9.31[a]		

[1]LRFI = Low residual feed intake; HRFI = high residual feed intake.

[a,b,c,d,e,f,g,h,i,j,k,l]Means within a column by dependent variable with different superscripts differ ($P < 0.05$). Winter storms occurred on 25 November, 30 November, and 13 December, 2015.

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Use of a human tri-axial pedometer for measurement of sheep activity

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ABSTRACT: The accuracy of a simple, human tri-axial pedometer at measuring sheep steps at a walk was investigated. Adult ewes (n = 10) were subjected to a three week halter-training program, with the end result of ewes being able to walk for 100 m next to a handler with little or no resistant behavior. A tri-axial pedometer was attached to the left hind leg. Ewes were led for 100 m and the number of steps reported by the pedometer was recorded. A handheld video camera was used to record each trip, and the visual step count of the ewe was determined from the video recording. Each ewe was led through this pattern twice, and the average of the pedometer and visual step count at 80 m was used for statistical analysis. A Wilcoxon signed rank test was used to compare the means of the pedometer and visual step counts, and a Pearson correlation was drawn. The means of the pedometer and visual step counts were statistically different ($P < 0.001$) and the correlation was negligible (r = 0.03). The simple tri-axial pedometers overestimated the amount of steps each ewe made, and therefore, cannot be considered accurate at measuring sheep activity.

Key words: sheep, activity, tri-axial pedometer

INTRODUCTION

Animal activity levels have been reported to be indicative of animal health and welfare (Moreau et al., 2009; Müller and Schrader, 2003). Recent evidence also suggests that there are differences in the activity level between efficient and inefficient animals, and that approximately 10% of variation in residual feed efficiency can be explained by differences in activity (Herd et al., 2004). Improving residual feed intake has been proposed as a method to decrease cost to producers (Richardson and Herd, 2004) and increase sustainability of food animal production (Capper, 2011). Lack of understanding regarding the variation induced by different patterns of activity is potentially inhibiting the ability of researchers to fully understand the physiological impacts of the use of residual feed intake as a selection tool. Many methods to measure activity in small ruminants such as sheep and goats have focused on obtaining distance measurements and behavior while grazing through the use of mileage recorders (Cresswell and Harris, 1959) and different types of activity sensors (Champion et al., 1997; Langbein et al., 1969; Moreau et al., 2009; Powell et al., 1968). Recently, improved technology for animal activity assessment has become available in the form of tri-axial activity sensors (Nielsen et al., 2010). Although there is considerable research in cattle investigating activity and resting patterns (Müller and Schrader, 2003; Nielsen et al., 2010; Robért et al, 2011), the methods used require expensive equipment, such as tri-axial sensors that are specifically made for ruminants, and/or in- depth computational programs and equations to smooth out and manipulate data. The expense of these products and computer software, as well as the task of manipulating data, can prohibit measurement of activity.

The objective of this study was to validate an inexpensive, human tri-axial pedometer without any attached computer software for use in sheep research as a relatively simple method to measure sheep activity, specifically step count at a walk, with the goal that simple, tri-axial pedometers would prove useful to unobtrusively measure activity level in lambs in the future.

MATERIALS AND METHODS

This experiment was conducted at the Montana State University Bozeman Agricultural Research and Teaching Farm (**BARTF**). Animal care, handling, and protocols used in this experiment were approved by the Montana State University Agricultural Animal Care and Use Committee.

Animals and halter training

Adult western white-faced ewes (n = 12), 2 to 6 years of age, with no previous halter training were randomly selected from the Montana State University flock maintained at the Red Bluff Research Ranch. Ewes were transported to the BARTF in February, 2014. Ewes had free access to water and were fed mixed-grass hay 2-3 times daily. Halter training began 24 hours after acclimation to the facility. Training sessions lasted no longer than 20 minutes per ewe, twice daily for 3 weeks, with a final goal of a 100 m walk on lead with little to no resistant behavior. Halter training was necessary to test the pedometer in a controlled manner. Two ewes did not acclimate to the halter and were removed from the study.

Pedometer Calibration

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Ozeri 4x Sport[3] Triaxial Pedometers (Ozeri USA, San Diego, CA) were attached to the left hind leg above the fetlock of each ewe using vet wrap, plastic bags, and duct tape (Fig. 1). A standard 10 m distance, as recommended by the manufacturer, was marked on the barn floor and each ewe was then allowed to walk at her own pace on the halter and lead through the 10 m distance twice. A step was defined as the forward movement of the hind leg with the attached pedometer. The step counts were averaged for each standard 10 m distance for each ewe. Average step length was calculated and used to determine individual ewe stride length. A pedometer was then calibrated based upon individual stride length for each ewe.

Pedometer Trials

A 50 m distance was measured and marked on the ground outside the barn. Ewes had been acclimated to the area that was used for the 50 m distance. Ewes were individually led down this path and back at a walk, for a total distance of 100 m. This procedure was repeated twice for each ewe. Ewes were allowed to walk at their own pace, just as they were during the calibration, unless resistant behavior occurred. If a ewe resisted, pressure was applied to the lead until she resumed walking; if a ewe began to trot or jump, pressure was applied to the lead until she slowed to a walk. The start and end count of the pedometer was recorded for step count. All trips were performed on the same day.

Each trip was visually documented using a Sony Handycam (Sony USA, New York, NY). Video data were analyzed by two independent observers to determine the visual step count of each ewe. A manual cell counter (Clay Adams, Parsippany, NJ) was used to count the number of steps for both 100 m trips of each ewe. Each video recording of a trip was analyzed for step counts twice per observer, and the visual step count for each ewe used for statistical analysis was the average of the four visual step counts of the observers.

Statistical Analysis

Video data were analyzed using a one-way ANOVA to determine if the visual step counts differed between observer, and variance was tested using Levene's test for homogeneity (SAS, Cary, NC, USA). There was no difference between observer ($P > 0.27$) for step count and variance was equal ($P > 0.82$). Visual step counts were pooled for analysis. To ensure precision of visual counts, only 80 m of the 100 m walk were counted, and the pedometer step count was reduced to 80% of the total count. The experimental unit was the individual animal. The independent variable was visual step count, and the dependent variable was the pedometer step count. Differences between the mean visual step count and mean pedometer step count were analyzed using Wilcoxon Signed Rank Test (SAS, Cary, NC, USA), and a Pearson product-moment correlation was determined for visual step count and pedometer step count (SAS, Cary, NC, USA). Means are reported (± SD). Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The mean pedometer step count of ewes was 166.8 ± 23.4; whereas, the mean visual step count for the same ewes was 93.3 ± 4.9. The Ozeri 4XSport[3] Triaxial Pedometer overestimated step count and was not accurate at predicting steps at a walk (Table 1; $P < 0.001$). Moreover, there was no correlation (r = 0.03) between the pedometer and visual step counts for these ewes. For each ewe, the pedometer step count was always greater than the visual step count.

Table 1. Wilcoxon Signed Rank Test means for pedometer and visual step counts for adult, western white-faced ewes

Item	Pedometer Step Count	Visual Step Count	*P*-value
n	10	10	
Steps	166.8 ± 23.4[a]	93.3 ± 4.9[b]	0.001

[a,b] Means within a row with different superscripts differ ($P \leq 0.05$).

Accurate measurement of the activity of animals has continuously been a subject of research. It is possible to accurately measure activity by analyzing video recordings and directly observing the animals, but these methods are very time consuming and require a large amount of labor (Müller and Schrader, 2003). Additionally, the presence of direct observers can cause deviation from normal behavioral patterns of activity (Robért et al., 2011).

A majority of the research on sheep activity involves measurements of grazing activity and distance traveled, not measurements of activity level, which was the objective of the current study. Previous measurement tools include a rangemeter (Cresswell and Harris, 1959), simple pedometers (Powell, 1968), solar powered activity-data-loggers (Langbein et al., 1996), and mercury tilt switch sensors (Champion et al., 1997). The rangemeter and mercury tilt sensors were considered to accurately measure distance traveled (Champion et al., 1997; Cresswell and Harris, 1959). Activity data loggers, used to measure wild mouflon sheep activity, were highly correlated to video measurements of activity (Langbein et al., 1996). In contrast to the other methods of measurement, Powell (1968) noted that the accuracy of simple pedometers was dependent on individual calibration of pedometers. They concluded that pedometers were not accurate for measurements of distance traveled in sheep.

Modern tri-axial accelerometers and pedometers have been proposed to be effective and accurate tools for activity measurement due to their small size (Robért et al., 2011). These can continually measure activity (Robért et al., 2011), and have a greater capacity to detect movement (Marais et al., 2014; Moreau et al., 2009). Not only do the tri-axial accelerometers detect movement in the vertical and horizontal dimensions, but forward and backward acceleration are captured in a second horizontal dimension (Moreau et al., 2009). Recently, the manipulation of tri-axial accelerometer data from sensors attached to sheep necks was reported to result in accurate measurement of

five patterns of sheep behavior, specifically walking, running, standing, lying, and grazing (Marais et al., 2014). Activity such as walking has typically been measured by pedometers and accelerometers placed on the hind leg (McGowan et al., 2007; Müller and Schrader, 2003; Nielsen et al., 2010; Robért et al., 2011); sensors placed around the neck are better suited to capture up/down grazing motion (Moreau et al., 2009).

The successful use of tri-axial pedometers and accelerometers in the aforementioned research does not correspond with the results of the current study, in that there was no relationship between the step counts from the tri-axial pedometer and the visual step count. The method used for visual step count has been used in many studies (Champion et al., 1997; Higginson et al., 2010; Langbein et al., 1996; Nielsen et al., 2010), and the pedometers used in the present study matched criteria suggested by Moreau et al. (2009) and Müller and Schrader (2003) for accurate measurements of activity level. The incongruity in results of the present study and previous studies utilizing tri-axial pedometers and accelerometers may have been a result of the use of the simple tri-axial recorders used in this study. By design, the pedometers utilized in the current study did not include any computer software to allow for filtering and manipulation of the data. The inaccurate step counts measured by the simple tri-axial pedometers indicate that data filtering and manipulation are required to accurately measure walking activity in adult, western white-faced ewes.

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BREEDING AND GENETICS

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Influence of first calving date on stayability in *Bos indicus* crossbred cows

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ABSTRACT: Longevity is one of the most important, complex, and difficult to improve traits sought by cow-calf producers. Consequently, a measurement or tool that could be utilized early in a cow's life to predict her future reproductive performance would be advantageous to producers and researchers alike. In this study, we sought to determine the effect of first calving season period on stayability in Nellore-crossbred females through 5 yr, 6 yr, and 7yr of age. Stayability through each age was scored as a binary trait, with 1 indicating the cow remained in the herd and 0 indicating she was culled, given either a perfect calving or weaning record. Each female was assigned a value of 1, 2, 3, or 4 corresponding to the respective 21-d period of her first calving season (for first, second, or third 21-d period, or > 63 d, respectively). Cow stayability models were evaluated through mixed model procedures (PROC MIXED in SAS). Of the cows with perfect calving records, more ($P < 0.05$) females that first calved in the first 21-d period remained in the herd than those that first calved in the second 21-d period through 5 yr (66.9% vs. 53.6%), 6 yr (60.0% vs. 45.9%), and 7 yrs of age (56.7% vs. 39.3%). They also differed ($P < 0.005$) from females whose first calf was born 63 d or later into the calving season through ages 5 (66.9% vs. 36.0%), 6 (60.0% vs. 29.5%), and 7 (56.7% vs. 27.2%). Of the cows with a perfect weaning record, more ($P < 0.05$) of the females that first calved in the first 21-d period of the calving season remained in the herd through 5 yr (56.1% vs. 31.0%) and 6 yrs of age (48.3% vs. 26.0%) than heifers whose first calf was born at the end of the calving season. These results document that regardless of the culling criteria, *Bos indicus* crossbred heifers that calve early in their first calving season are more likely to maintain a perfect calving or weaning record later in life than females that calve late in the first calving season. Consequently, there is potential that the heifer's first calving season period may be used as valuation or culling criteria when selecting for stayability and longevity, or when merchandizing beef replacements.
Keywords: beef cows, calving season, longevity, Nellore, stayability

INTRODUCTION

Longevity is one of the most important, complex, and difficult to improve traits sought by producers. Beef cow stayability is defined as a cow's ability to remain productive in the herd and maintain a perfect reproductive record to the age of 6 (Snelling et al., 1995). In the current beef industry, stayability is often used as a measure of a cow's potential longevity and serves as a critical benchmark for many producers, as a cow must stay within the herd long enough to offset her costs of production and become profitable.

Longevity typically has a large influence on profitability. Maintaining productive cows in the herd for more time has been shown to generate economic returns ranging by breed from $118 to $244 per cow for each additional year of productivity, and to increase cow value (Garcia et al., 2014). Furthermore, a longer productive life offsets the initial costs of developing replacement heifers (Endecott et al., 2013). *Bos indicus* x *Bos taurus* crossbred cows are recognized as having long reproductive lifespan, likely due to the added benefits of heterosis and exceptional adaptation to the climates in which they are raised (Riley et al., 2001, Thrift et al., 2003). Additionally, it has been shown that heifers that calved in the first 21 days of their first calving season were more likely to have a perfect calving record until 10 yrs of age than heifers that calved in the following 21-day intervals (Mousel et al., 2012).

As with any reproductive trait, stayability is an expensive and complex trait to measure. Consequently, a measurement or tool that could be utilized early in a cow's life to predict longevity would be advantageous to producers and researchers alike. If a cow's initial reproductive performance could be indicative of her subsequent stayability potential, her value as a replacement could be adjusted, or perhaps the unnecessary costs of maintaining her in the herd may be negated. In this study, we sought to determine the effect of first calving season period on stayability in Nellore-crossbred females.

MATERIALS AND METHODS

Cows in this study were Nellore-Angus F_2 crosses, Nellore-Angus x Brahman-Angus crosses, or Nellore-Angus x Brahman-Hereford crosses; all females were sired by 4 Nellore x Angus F_1 bulls. The F_2 females were produced through ET, and others were produced through natural service (NS) mating. These females were born in 2003 through 2007 at the Texas A&M AgriLife Research Center at McGregor, TX. Spring-born heifers (ET and NS) were bred to calve first at 2 yr of age. Fall born heifers (ET only) were allowed the opportunity to breed to first calve at 2 yr of age the following fall, and those that failed to initially conceive were bred to first calve at 2.5 yr of age, without a skip counted against them, and then maintained on a Spring-calving schedule. Fall-born heifers that first calved at 2 yr of age were held through the winter without mating opportunity and rebred the following spring/summer to be on a Spring-calving

schedule, with their second calf born at 3.5 yr of age. All females were subsequently managed for Spring-calving only. The actual culling criteria used are that females are allowed to remain in the herd until failure to wean their second calf, until 12 yr of age. As a result, females born within any birth year-season may have first calved at 2 yr, or 3 yr (for spring-calved heifers), or 2 yr, 2.5 yr, 3 yr, or 3.5 yr (for fall-calved heifers). Ages on the Fall-born females were rounded down for age category designations (4.5 yr considered 4 yr old, etc.).

In the present study, cow stayability through 5 yr, 6 yr, and 7 yr of age was evaluated. This trait was evaluated under two imposed culling protocols. First, was removal of all ensuing records when a cow was first diagnosed as non-pregnant when checked for pregnancy or upon her first failure to calve (perfect calving record). Second, was removal of subsequent records after one failure to calve or one failure to raise a calf to weaning (perfect weaning record). Stayability through each age was scored as a binary trait, with 1 indicating the cow remained in the herd, and 0 indicating she was culled. Each female was described with a value of 1, 2, 3, or 4 corresponding to the respective 21-d period of their first calving season (for first, second, or third 21-d period, or > 63 d, respectively). Cow stayability models for perfect calving record (n = 280) and perfect weaning record (n = 285) were evaluated through mixed model procedures (PROC MIXED in SAS) and included cow birth year-season, cow breed, and first calving season period as fixed effects and sire of cow as a random effect. When *F*-tests indicated differences, least squares means were compared through *t*-tests. From these analyses, cow stayability least squares means represent the proportion of cows at each age that remained in the herd. Due to the nature of this dataset, age at first calving was confounded with female birth year-season; however, frequency summary tables for age at first calving were evaluated. Cow age in days at first calving (range of 671 to 802 d for 2 yr olds and 822 to 955 for 2.5 yr olds) was investigated for potential differences across calving period, but no differences within age category were observed.

RESULTS AND DISCUSSION

Cow birth year season accounted for significant differences in cows with a perfect calving record through 5, 6, and 7 yrs of age, and in cows with a perfect weaning record through 5 and 6 yrs of age. However, the breed composition of the female did not affect any of these traits, including cows with a perfect weaning record through 7 yrs of age. Age category (2 yr, 2.5 yr) at first calving was confounded with birth year-season. Table 1 shows the distribution of age in yr at first calving across first calving season period and perfect calving records through 5 yr of age. There were no females that first calved at 3 or 3.5 yr that remained in the herd past 5 yr of age, under the imposed culling criteria.

Cow stayability through 5 yr, 6 yr, and 7 yr of age (which were the ages available on project cows at the time of analyses) was evaluated for all cows under the imposed culling procedures. Of the females with perfect calving records (Table 2), there was a proportion of heifers that calved in the first 21 d of their first calving season that were able to maintain a perfect calving record through 5 yr of age (0.669 vs. 0.536), through 6 yr of age (0.600 vs. 0.459), and through 7 yr of age (0.567 vs. 0.393), compared to ($P < 0.05$) those heifers that calved within the second 21-d of the season. Substantially more ($P < 0.005$) of these early calving heifers remained in the herd than their contemporaries that first calved at the very end of the season through 5 yr of age (0.669 vs. 0.360), 6 yr of age (0.600 vs. 0.295), and 7 yr of age (0.567 vs. 0.272). In the analysis of cows with a perfect weaning record (Table 3), the proportion of heifers that calved in the first 21-d period of the calving season that maintained a perfect weaning record through 5 yrs of age (0.561 vs. 0.310), and through 6 years of age (0.483 vs. 0.260), was greater ($P < 0.05$) than heifers whose first calf was born 63 d or later into the calving season. Results of this study with *Bos indicus-Bos taurus* crossbred females correspond to those of Mousel et al. (2012). Those authors reported average longevity of 8.2 yr, 7.6 yr, and 7.2 yr for USMARC heifers that calved in the first, second, and later 21-d periods, respectively of their first calving season, and, longevity of 5.1 yr and 3.9 yr for South Dakota heifers that calved in the first or later 21-d period, respectively, of their first calving season (Mousel et al., 2012).

Our results indicate that regardless of the culling criteria, *Bos indicus* crossbred heifers that calve early in their first calving season are more likely to remain in the herd through 5 yr, 6 yr, and 7 yr of age than heifers that first calve late in the season, which correspond with observations in *Bos taurus* females. It is likely that calving early in the first calving season gives heifers the opportunity for additional recovery time before the start of the breeding season, increasing their likelihood of breeding back the next year. Our results also showed only small changes between years 5, 6, and 7, suggesting that if a cow were to experience either calving or weaning failure, it was likely to occur before 5 yr of age. This suggests that a heifer's ability to conceive earlier in the breeding season and then give birth early in the calving season directly relates to her potential for stayability, and thus increased lifetime productivity. Consequently, there is potential that the heifers' first calving season period may be used as valuation or culling criteria when selecting for stayability and longevity, or when merchandizing beef replacements. While it could be speculated that this may be a result of the earlier onset of puberty and first estrus in more fertile, reproductively sound females, additional research will be necessary to elucidate the underlying causes of this phenomenon. It is a long term goal of the present study to evaluate longevity and lifetime productivity on these females and to investigate genomic regions influencing these types of traits.

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Table 1. Distribution of first calving season period and perfect calving records through 5 yr age relative to the female age at first calving.

	First calving season period					Calving record		
Age at first calving (yr)	First 21 d	Second 21 d	Third 21 d	> 63 d	Subtotal (n)	0	1	Subtotal (n)
2	34.6	37.8	15.4	12.2	188	33.7	66.3	193
2.5	45.6	36.8	14.0	3.5	57	32.1	67.9	56
3	42.1	42.1	5.3	10.5	38	100	0	38
3.5	0.0	33.3	33.3	33.3	3	100	0	3
Subtotal (n)	107	109	40	30	286	124	166	290

Table 2. Proportion of females with perfect calving records at 5, 6, and 7 yr of age based on calving period of their first calving season

Cow age	First 21 d	Second 21 d	Third 21 d	Over 63 d
5 yr	$0.669^{a} \pm 0.0563$	$0.536^{b} \pm 0.0613$	$0.514^{a,b} \pm 0.0856$	$0.360^{b} \pm 0.0957$
6 yr	$0.600^{a} \pm 0.0550$	$0.459^{b} \pm 0.0601$	$0.454^{a,b} \pm 0.0852$	$0.295^{b} \pm 0.0956$
7 yr	$0.567^{a} \pm 0.0556$	$0.393^{b} \pm 0.0607$	$0.419^{a,b} \pm 0.0861$	$0.272^{b} \pm 0.0966$

$^{a, b}$ Means within row with different superscripts differ ($P \leq 0.05$).

Table 3. Proportion of females with perfect weaning records at 5, 6, and 7 yr of age based on calving period of their first calving season

Cow age	First 21 d	Second 21 d	Third 21 d	Over 63 d
5 yr	$0.561^{a} \pm 0.0559$	$0.445^{a,b} \pm 0.0611$	$0.436^{a,b} \pm 0.0858$	$0.310^{b} \pm 0.0966$
6 yr	$0.483^{a} \pm 0.0548$	$0.383^{a,b} \pm 0.0600$	$0.388^{a,b} \pm 0.0850$	$0.260^{b} \pm 0.0959$
7 yr	0.438 ± 0.0543	0.335 ± 0.0595	0.371 ± 0.0844	0.254 ± 0.0952

$^{a, b}$ Means within row with different superscripts differ ($P \leq 0.05$).

EXTENSION EDUCATION

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Survey of serum trace mineral concentrations in weaned Montana ram lambs

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ABSTRACT: Clinical and subclinical trace mineral deficiencies can limit productivity in western sheep production systems. The objective of the study was to quantify trace mineral status among Montana ram lambs post weaning. Based on prior research investigating forage trace mineral concentrations and trace mineral status in cattle, we hypothesized that clinical and subclinical deficiencies would be most prominent with Zn and Se. To test this hypothesis, serum samples (n = 201) were collected from ram lambs 8 to 10 mo of age (BW 52.8 ± 16 kg) at 21 locations throughout Montana and analyzed for Co, Cu, Fe, Mn, Mo, Se, and Zn. The average concentration and range for each trace mineral analyzed in the serum samples were: Co (1.00 ± 0.079 ng/mL; 0.09-6.22 ng/mL), Cu (0.84 ± 0.016 µg/mL; 0.3-1.61 µg/mL), Fe (154.85 ± 3.682 µg/dL; 26-350 µg/dL), Mn (2.56 ± 0.225 ng/mL; 0.7-31.3 ng/mL), Mo (40.14 ± 5.001 ng/mL; 2.8-456.5 ng/mL), Se (111.42 ± 3.31 ng/mL; 16-197 ng/mL), and Zn (0.73 ± 0.015 µg/mL, 0.3-1.74 µg/mL). The two most deficient and marginally deficient minerals across Montana were Se (19% of ranches deficient; 24% of ranches marginally deficient) and Zn (14% of ranches deficient; 52% of ranches marginally deficient). All Se deficient samples were obtained from western Montana. There was considerable variation in serum trace mineral concentrations within individual flocks. Descriptive statistics were analyzed using SAS. Given that Se and Zn play major roles in growth, fertility, and immunity, results suggest opportunities for more effective supplementation strategies. Producers and nutritionists alike can use these results to identify mineral deficient areas and develop cost effective mineral supplementation management practices.

Key words: trace minerals, zinc, selenium, Montana, sheep, ram lambs

INTRODUCTION

Forage mineral concentrations are variable throughout regions of the United States (Mortimer et al., 1999; Mathis et al., 2004;). Seasonality, climate, plant maturity, along with other factors affect mineral availability in forages/feed, which are the main source of minerals, except in circumstances where there are excessive mineral concentrations in water (NRC, 2007).

Support for this study was provided by the National Sheep Industry Improvement Center. The authors would also like to express appreciation to Weston Helle, Monica Ebert and the ranches that allowed access to their sheep.

With over 147,040 square miles of diverse geographic makeup, the variability of trace mineral concentration of Montana's forage is undoubtedly diverse. Montana has an estimated 230,000 sheep and lambs, 210,000 of those are breeding sheep ranking Montana 7th in breeding sheep numbers in the United States (USDA-NASS, 2016). Even with a large sheep population and general knowledge of trace mineral deficiencies, specific research attempting to quantify trace mineral status in Montana sheep populations has not previously been examined.

The objective of this study was to quantify trace mineral status in ram lamb sub-populations to identify deficiencies during early post weaning periods of ram lamb development. We hypothesized that clinical and subclinical deficiencies are most prominent in regards to Se and Zn, based on prior research investigating forage trace mineral concentrations (Mortimer et al., 1999) and trace mineral status in Montana range cattle (Ricketts et al., 2002).

MATERIALS AND METHODS

Methods described herein were approved by the Montana State University Institutional Animal Care and Use Committee.

From September 24, 2015 to November 23, 2015, serum samples were collected. Montana was divided into an east and west region at 48.5833˚ N longitudinal line. Ranches (n=21) involved in the survey were from a wide range of environments, 11 from the west and 10 from the east. The locations spanned from Dillon, Mt (45.2158˚ N, 112.6342˚ W) to Wolf Point, MT (48.0914˚ N, 105.6425˚ W), representing a distance of approximately 805 km (**Figure 1)**.

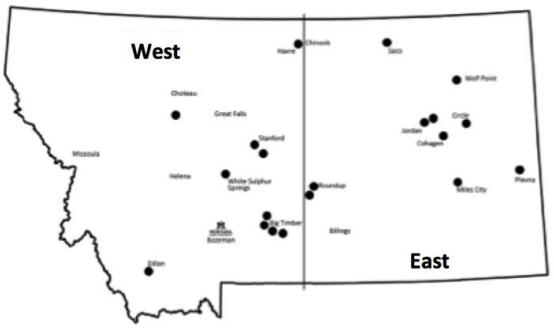

Figure 1: Map of sampling locations and longitudinal division of East and West regions

Ranches were chosen that currently had ram lamb populations being developed for breeding purposes.

Blood samples were drawn within 2 mo post weaning when the approximate age of the animals were 8 to 10 mo (BW 52.8 ± 16 kg). Breed composition of the rams included Targhee (n = 95), Rambouillet (n = 27), Columbia (n = 20), South African Meat Merino (n = 1), Suffolk (n = 12), Hampshire (n = 15), Debouillet (n = 2), Merino (n = 2), and various crosses (n = 27). A total of 340 serum samples were initially collected across the 21 locations, however 201 serum samples were randomly subsampled for analysis thereafter to reflect a minimum of 15% of the ram lamb population at each ranch location.

All blood samples were collected via jugular venipuncture into 13 × 100 mm trace mineral royal blue top vacutainer tubes (Covidien, Mansfield, MA) without any additives. Samples were put on ice until they were centrifuged at 2800 rpm for 15 min to decant serum, and 2 aliquots were taken from each sample. Aliquots were kept at -20˚C until sent for analysis at Michigan State University Diagnostic Center for Population and Animal Health. Serum trace mineral analysis included Co, Cu, Fe, Mn, Mo, Se, and Zn concentrations. Ranch (n = 21) was the experimental unit and data was analyzed using the MEANS, UNIVARIATE and FREQ procedures of SAS.

RESULTS AND DISCUSSION

Reference ranges for sheep serum trace mineral concentrations were provided by T. Herdt at the Michigan State University Diagnostic Center for Population and Animal Health (Herdt, 2000). Results from the survey suggested adequate serum trace mineral concentrations for Co, Cu, Fe, and Se (**Table 1**). Average serum Mn and Mo concentrations (**Table 1**) were greater than adequate (> 2 and 30 ng/mL), but were not determined to be toxic. Average serum Zn concentrations were found to be deficient (< 0.8 µg/mL) in Montana ram lambs.

Cobalt. Co concentrations were adequate, with average and range 1.00 ± 0.079 ng/mL and 0.09-6.22 ng/mL. A concentration of 0.1 ng/mL and above is thought to be adequate in ram lamb serum samples

Copper. Cu serum concentration average and range was 0.84 ± 0.016 µg/mL and 0.3-1.61 µg/mL. The average was within an adequate range 0.7-2.0 µg/mL. Authors acknowledge that liver biopsies would have been a superior indicator of Cu status (Herdt, 2000) but due to the collection of samples from privately owned sheep, liver biopsies were not feasible.

Iron. Fe serum concentration average and range was 154.85 ± 3.682 µg/dL and 26-350 µg/dL, with an adequate range being between 116-222 µg/dL. Fe is the most abundant trace mineral in the body and approximately 60% of it is found in hemoglobin as an essential part to oxygen and carbon dioxide transportation (NRC, 2007)

Manganese. Mn serum concentration average and range was 2.56 ± 0.225 ng/mL and 0.7-31.3 ng/mL. An adequate range for serum Mn is between 0.5-2.0 ng/mL. Although the Mn serum concentration average across the state is higher than what is found to be adequate concentration range, Mn is low in toxicity even at high levels (NRC, 2007). An antagonistic relationship exists between Fe and Mn, enabling a minimum level of Mn to reduce appetite and growth rate in some weaned lambs (NRC, 2007)

Molybdenum. Mo serum concentration average and range was 40.14 ± 5.001 ng/mL and 2.8-456.5 ng/mL. Mo serum concentrations have been reported to range between 12-30 ng/mL in healthy sheep, however accurate reference ranges have only recently been established (T. Herdt, MSU Diagnostic Center for Population and Animal Health personal communication). A larger database is needed to determine true adequate ranges and make accurate comparisons to the current data set.

Selenium. Se serum concentration average and range was 111.42 ± 3.31 ng/mL and 16-197 ng/mL, with an adequate range of 110-160 ng/mL. Selenium status across MT ranches is shown in **Figure 2**. Results suggest the mean serum selenium concentrations are within adequate reference ranges yet approximating marginal status. Clinical signs of Se deficiency are often manifest as nutritional myopathy (white muscle disease,) but can also result in production losses in subclinical instances. Marginal deficiencies can cause impairments in growth performance, loss of milk yield, decreased reproductive performance and loss of wool production but can be remedied with Se supplementation (Slen et al, 1961; Gabbedy, 1971; McDonald, 1975; Suttle, 2010).

The ranches from the eastern half of Montana

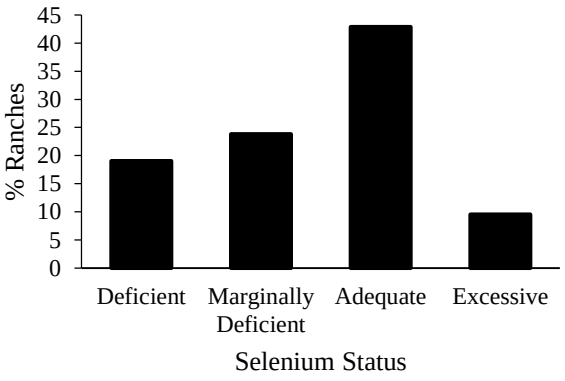

Figure 2: Distribution of Se status across 21 Montana sheep operations. Deficient: < 50 ng/mL; Marginally deficient: 50 to 90 ng/mL; Adequate: 110 to 160 ng/mL; and Toxic: > 160 ng/mL (Herdt, 2000).

were 0% deficient, 10% marginally deficient, 60% adequate, and 30% excessive based on average serum Se concentrations. Ranches in the western half of Montana were 36.4% deficient, 36.4% marginally deficient, 27.3% adequate, and 0% in excess based on average serum Se concentrations. Montana as a whole had 100% of Se deficient cases and 80% of marginally deficient ranches occur in the western half of the state.

Zinc. Zinc serum concentration average and range was 0.73 ± 0.015 µg/mL and 0.3-1.74 µg/mL with an adequate range of 0.8-1.2 µg/mL. Zinc status of ram lambs is located in **Figure 3**. Zinc is difficult to analyze because there is no one well-defined storage area in the

body but serum levels seem to be the best indicator of Zn status in the animal (Herdt et al., 2000). Zinc is the next abundant trace mineral in the body second to iron (Herdt et al., 2000). Therefore, Zn plays significant roles in the immune system, reproductive capabilities, and growth characteristics by influencing enzyme activity and gene expression of proteins (NRC, 2007).

The ranches from the eastern half of Montana were 20% deficient, 50% marginally deficient, 30% adequate, and 0% excess based on average serum Zn concentrations. The western half of Montana ranches were 9.1% deficient, 54.6% marginally deficient, 36.4% adequate, and 0% in excess based on average serum Zn concentrations. Montana as a whole had 66% of Zn deficient and 45.5% of Zn marginally deficient ranches found on the eastern half of the state. Approximately 2/3 of ranches sampled were categorized as deficient or

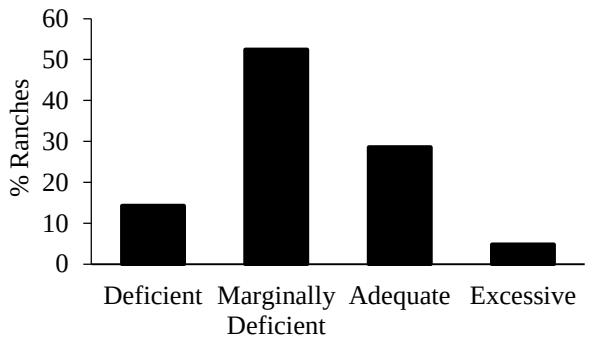

Figure 3: Distribution of Zn status across 21 Montana sheep operations. Deficient: < 0.6 µg/mL; Marginally deficient: 0.6 to 0.8 µg/mL; Adequate: 0.8 to 1.2 µg/mL; and Toxic: > 1.2 µg/mL (Herdt, 2000).

marginally deficient in serum Zn concentration. Studies have shown that variability in mineral consumption exists in flocks that are provided mineral ad libitum (Ragen et al., 2015). This could account for the variability that we witnessed within flocks in this study. Surprisingly, 20% of ranches sampled described their mineral supplementation strategies as inconsistent, and sporadic throughout the year. Deficiencies and marginal deficiencies in trace mineral status may be a result of both mineral inclusion rate, and bioavailability of the chemical form in the mineral supplement (oxide vs. chelated source), and delivery method (block vs. granulated). Mineral content of water sources and ram trace mineral status is currently being evaluated to identify antagonistic relationships across ranches sampled.

IMPLICATIONS

Trace mineral deficiencies exist among ram lamb populations in Montana and should be taken into account when determining sheep management practices and mineral supplementation strategies. Variability exists among individual flocks, likely because of varied consumption and other factors. On average selenium levels were lower in animals reared in western Montana, while zinc was lower in animals sampled from operations located in the eastern half of the state.

Future research will look at the most effective and economical way to adequately supplement essential trace minerals for sheep populations across Montana with an immediate emphasis on Zn source an it's effects on growth, fertility, and immune function in developing rams.

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Table 1. Minimum, maximum, mean, median and standard error of serum trace mineral concentrations from Montana ram lambs (n = 201)

Trace Mineral	Minimum	Maximum	Mean	Median	Standard Error
Se, ng/mL	16.00	197.00	111.42	122.00	3.310
Zn, µg/mL	0.30	1.74	0.73	0.71	0.015
Co, ng/mL	0.09	6.22	1.00	0.50	0.079
Cu, µg/mL	0.30	1.61	0.84	0.80	0.016
Fe, µg/dL	26.00	350.00	154.85	149.00	3.682
Mn, ng/mL	0.70	31.30	2.56	1.80	0.225
Mo, ng/mL	2.80	456.50	40.14	15.40	5.001

Note: Reference ranges to determine adequacy for sheep serum trace mineral concentrations were provided by T. Herdt at the Michigan State University Diagnostic Center for Population and Animal Health. Co (≥ 0.1 ng/mL), Cu (0.7-2.0 µg/mL), Fe (116-222 µg/dL), Mn (0.5-2.0 ng/mL), Mo (12-30 ng/mL), Se (110-160 ng/mL), and Zn (0.8-1.2 µg/mL).

FORAGES AND PASTURES

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Methods to increase productivity of spring calving production systems in the Nebraska Sandhills

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ABSTRACT: A 2-yr study evaluated effects of late-gestation supplementation, post-partum progestin administration, and creep feeding on productivity of March calving cows. In yr 1, 120 crossbred cows (BW 479 ± 57 kg) were assigned to 1 of 4 levels of late-gestation supplementation, 1 of 2 levels of post-partum progestin, and 1 of 2 levels of creep feed in a 4 x 2 x 2 factorial arrangement of treatments in a completely random design. The four supplement levels fed were: 1) 0 kg/d Dec 1 to Mar 1; 2) 0.41 kg/d DM Dec 1 to Mar 1; 3) 0.41 kg/d DM Jan 15 to Mar 1; or 4) 0.82 kg/d DM Jan 15 to Mar 1 while cows grazed dormant upland range. Levels of exogenous progesterone post-partum: 1) controlled internal drug release device for 7 d; or 2) no progesterone administration. Creep feed levels were: 1) unrestricted access by the calf to creep feed which contained an intake limiter; or 2) no access to creep feed. The higher levels of late-gestation supplementation increased cow BW ($P < 0.05$) and increased cow BCS ($P < 0.05$) but did not affect ($P > 0.12$) reproductive measures or calf performance. Exogenous progesterone administration post-partum did not affect ($P > 0.13$) cow or calf performance. Allowing calves access to creep feed increased ($P < 0.01$) calf BW at weaning by 21 kg.

Key Words: creep feed, progesterone, supplement

INTRODUCTION

Extending the grazing season to include grazing dormant pasture decreases production costs (Adams et al., 1994). Research has determined supplemental RDP is necessary to maintain BCS of gestating cows grazing winter range in the Nebraska Sandhills (Hollingsworth-Jenkins et al., 1996). Feeding supplement to cows grazing winter range during the last trimester of gestation has been shown to increase calf BW at weaning (Stalker et al., 2006; 2007) but it is not known if the timing of supplement feeding optimized progeny performance. Under-nutrition during gestation causes suboptimal conditions in the maternal uterine environment, which translate into depressed progeny performance (Wu et al, 2006). Potential cost savings could be achieved if the supplement amount and the duration supplement it is fed were reduced. Further efficiency might be achieved if supplemental feed delivered directly to the calf could undo the detrimental effects of undernutrition during gestation.

Administration of exogenous progesterone can shorten the post-partum interval (Lamb et al., 2008). If weaning occurs at a constant d for all calves in a herd, those born to cows with a shorter post-partum interval will be older and therefore weigh more than contemporaries born to cows that become pregnant later in the breeding season.

Objectives of this study were to determine effects of late-gestation supplementation, post-partum progestin administration, and creep feeding on productivity in spring calving systems.

MATERIALS AND METHODS

All procedures and facilities were approved by the University of Nebraska-Lincoln, Institutional Animal Care and Use Committee (Project 921). A 2-yr experiment used 120 crossbred (¾ Red Angus, ¼ Simmental), March calving cows (initial BW = 479 ± 57 kg) at the Gudmundsen Sandhills Laboratory, near Whitman, Nebraska, USA (lat 42.08° N, long 101.45° W, elevation 1073 m). Cows were stratified by BW within age and treatments were assigned randomly in a 4 x 2 x 2 factorial arrangement: 1) 0 kg/(cow • d) Dec 1 to Mar 1 (**DM0**), 0.41 kg DM/(cow • d) Dec 1 to Mar 1 (**DM1**), 0.41 kg DM/(cow • d) Jan 15 to Mar 1 (**JM1**), or 0.82 kg DM/(cow • d) Jan 15 to Mar 1 (**JM2**) of supplement (32% CP; 89% TDN); 2) administration of exogenous progesterone post-partum via a controlled internal drug release device (EAZI-Breed CIDR insert containing 1.38 g of progesterone; Zoetis Inc., Florham Park, NJ) for 7 d and prostaglandin $F_{2\alpha}$ (5 mL Lutalyse, Zoetis Inc.) administered on d seven (**CIDR**), or no progesterone administration (**NoCIDR**); and 3) unrestricted access by the calf to creep feed which contained an intake limiter (Accuration, Purina Animal Nutrition LLC, Gray Summit, MO) from July 15 to Nov 1 (**Creep**) or no access to creep feed (**NoCreep**). The study began in December when cows were located to 1 of 8 upland range pastures (35 ha) where supplement treatments were delivered on a pasture basis 3 d/wk until March 1. Beginning March 1 cows were managed as a single group and fed hay until the end of the calving season. On May 28 CIDR inserts were administered to cows assigned to the CIDR treatment. On June 4 CIDR inserts were removed and cows were administered prostaglandin $F_{2\alpha}$. All cows were exposed to fertile bulls (1:25 bull:cow ratio) for 45 d, with breeding season ending July 15. The non-creep treatment occupied 1 pasture and creep treatments occupied 2 separate pastures, for a total of 3 pastures. Creep treatment were introduced into pastures containing creep feeders surrounded by panels with openings sufficient to admit calves but prevent cow entry (8 openings, 38 cm wide).

Cow BW and BCS were measured at the beginning and end of the supplementation period pre-breeding and at weaning. Calf BW was measured at birth, the start of the breeding season, and weaning.

Cows were removed from the study for failure to wean a calf or become pregnant and were not replaced. Therefore, the

number of cows decreased throughout the 2 yr of data collection. Cows external to the experiment were introduced into pastures to maintain constant stocking rates for each pasture during experiment.

Cows assigned to the same winter supplement, CIDR and creep treatment within winter pasture served as the experimental unit. Replicated treatment means within yr were used for analyses of cow and calf response variables. Model fixed effects included winter supplement treatment, CIDR treatment, creep treatment and all possible interactions. Year and residual error were included in the model as random effects. Data were analyzed with the GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, NC). Effects of treatment were considered significant when $P < 0.05$ as detected by Fischer's test. When the F-test was significant, least square means of treatments were separated using a t-test when $P < 0.05$. There were no interactions ($P > 0.18$) among treatments; therefore, data are reported as main effects.

RESULTS AND DISCUSSION

Regardless of supplement amount offered, there was a notable fluctuation from initial cow BW to BW at weaning. Cows assigned to the DM0 treatment had the greatest differences in BW from start to finish. The greatest loss in BW occurred during the period between start of calving (March) to start of breeding (May) for all 4 treatments. Treatments fed supplement maintained BW. Differences in BW among supplement treatments were most notable at the start of the breeding season where DM0 cows had the lightest ($P < 0.05$) BW, JM1 and JM2 cows intermediate, with DM1 cows having the heaviest BW. Cow BCS was lower ($P < 0.05$) at the start of the breeding season for cows not supplemented than for cows assigned to DM1 and JM2 treatments, with JM1 cows being intermediate. Differences in BW and BCS caused by the supplementation treatment did not affect measures or reproductive efficiency such as calving date ($P = 0.16$), calving rate ($P = 0.58$), weaning rate ($P = 0.51$), and pregnancy rate ($P = 0.26$). Previous research examining effects of supplement fed to cows grazing winter range has demonstrated decreased weaning rate in cows not fed supplement in some studies (Stalker et al., 2006) but no effects in others. (Stalker et al., 2007; Rolfe et al., 2011). Supplement treatment did not affect calf BW at birth ($P = 0.12$), at beginning of dam's breeding season ($P = 0.46$), or at weaning ($P = 0.92$). Similar research (Stalker et al., 2006; Stalker et al., 2007; Rolfe et al., 2011) has consistently demonstrated decreased BW at weaning of calves born to cows not fed supplement during winter. Similar BW at weaning between calves born to cows not fed supplement and those fed supplement in this experiment was not expected. An additional yr of data may be needed to draw definitive conclusions.

Whether or not cows were administered a CIDR did not affect ($P > 0.13$) BW, BCS, reproductive measures, or calf BW. Exogenous progesterone was not expected to affect cow BW or BCS. Potential increased calf age and therefore, increased BW at weaning as a result of earlier conception in the breeding season due to progesterone administration was not realized ($P = 0.83$). Allowing calves access to creep feed increased ($P < 0.05$) calf BW at weaning by 21 kg. Total amount of creep that disappeared from feeder was 1.2 kg DM/(calf • d).

IMPLICATIONS

While feeding supplement increases cow BW and BCS it may not affect reproduction or calf performance, thus increasing production costs without increasing returns. Using a CIDR to shorten the post-partum interval in cow herds with existing acceptable reproductive performance may also increase costs without increasing returns. Feeding creep feed to calves is an effective means of increasing weaning BW but should be considered within the context of a cost/benefit analysis. Additional yr of data collection may be necessary to draw definitive conclusions.

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Table 1. Effects of winter supplement[1], post-partum progesterone administration[2], and calf access to creep feed[3] on cow body weight, body condition score (BCS), calving date, calving rate, weaning rate, pregnancy rate, and calf body weight

	Supplement				Progesterone		Calf feed			P-Value		
	DM0	DM1	JM1	JM2	CIDR	No CIDR	Creep	No Creep	SE[4]	Supp	Progest	Feed
Cow BW, kg												
Initial (Dec)	476	489	478	473	475	483	478	480	8	0.06	0.07	0.61
Calving (Mar)	450[c]	498[a]	469[b]	473[b]	472	473	466	479	7	< 0.01	0.91	0.03
Breeding (May)	431[c]	464[a]	445[b]	449[b]	444	450	444	451	5	< 0.01	0.22	0.16
Weaning (Nov)	477[b]	500[a]	484[b]	484[b]	483	489	489	484	15	0.02	0.24	0.29
Cow BCS[5]												
Initial (Dec)	4.9	5.0	5.0	5.0	5.0	5.0	5.0	5.0	0.1	0.78	0.80	0.39
Calving (Mar)	4.8[b]	5.2[a]	5.0[b]	5.2[a]	5.0	5.1	5.0	5.1	0.1	< 0.01	0.45	0.73
Breeding (May)	4.5[b]	4.9[a]	4.7[ab]	4.8[a]	4.7	4.7	4.7	4.7	0.1	< 0.01	0.48	0.33
Weaning (Nov)	5.2	5.2	5.3	5.4	5.3	5.3	5.3	5.2	0.2	0.33	0.62	0.51
Calving date[6], d	82	88	86	83	83	87	86	84	2	0.16	0.13	0.31
Born in 21 d[7], %	0.80	0.70	0.82	0.84	0.80	0.78	0.74	0.84	0.06	0.34	0.68	0.08
Calving rate[8], %	0.97	1.00	0.98	0.98	0.98	0.98	0.98	0.99	0.1	0.58	1.00	0.32
Weaning rate[9], %	0.94	0.98	0.95	0.97	0.95	0.97	0.95	0.97	0.1	0.51	0.58	0.44
Pregnancy rate[10], %	0.86	0.96	0.91	0.88	0.90	0.91	0.92	0.89	0.1	0.26	0.77	0.43
Calf BW, kg												
Birth (Mar)	35	36	34	35	35	35	35	35	1	0.12	0.61	0.22
Breeding (May)	74	73	70	74	73	72	72	74	2	0.46	0.60	0.17
Weaning (Nov)	237	234	235	237	235	236	246	225	7	0.92	0.83	< 0.01

[1]DM0: 0 kg/(cow • d) Dec 1 to Mar 1; DM1: 0.41 kg DM/(cow • d) Dec 1 to Mar 1; JM1: 0.41 kg DM/(cow • d) Jan 15 to Mar 1; JM2: 0.82 kg DM/(cow • d) Jan 15 to Mar 1.

[2]CIDR: CIDR insert containing 1.38 g of progesterone for seven d and prostaglandin $F_{2\alpha}$ administered on d 7 from May 28 to June 4.

[3]Creep: unrestricted access by the calf to creep feed which contained an intake limiter from July 15 to Nov 1.

[4]Standard error of the least squares mean (n = 4 observations per treatment replication [2/yr]).

[5]Scale of 1 (emaciated) to 9 (extremely obese).

[6]Day of yr calving occurred where January 1 = d 1.

[7]Cows calving within 21 d calculated by finding difference between birth date and breeding date and subtracting from 285.

[8]Calving rate calculated by dividing the number of cows to calve by the number of cows at the beginning of the production yr.

[9]Weaning rate calculated by dividing the number of cows to wean a calf by the number of cows at the beginning of the production yr.

[10]Pregnancy rate calculated by dividing the number of cows determined pregnant by the number of cows at the beginning of the production yr.

[abc]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

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Influence of supplement type and monensin addition on utilization of low-quality, cool-season forage by beef cattle

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ABSTRACT: Two studies were conducted to evaluate the influence of supplement composition and monensin addition on intake and digestibility of a low-quality (4.5% CP), cool-season forage, as well as cow performance. Treatments included a non-supplemented control (CON), approximately 30% CP supplements consisting of corn and urea (CU), CU + monensin (200 mg/day; CU+M), dried distiller's grains (DDGS), or DDGS + monensin (200 mg/day; DDGS+M). In Experiment 1, 5 steers were used in an incomplete 5 x 4 Latin square with four 28-d periods to compare the effects of monensin and supplement type on forage intake, digestibility and ruminal fermentation characteristics. Forage intake tended to be greater with supplementation ($P = 0.06$), was greater with DDGS compared with CU ($P = 0.03$), and was decreased with monensin addition ($P = 0.04$). Ruminal pH was increased with monensin; however, it was increased more with monensin addition to the DDGS supplement compared with the CU supplement ($P < 0.01$). In Experiment 2, 80 late gestation cows were stratified by age, BCS, and BW and randomly allotted to treatments (20 pens; 4 cows/pen; 4 pens/treatment). Pre-calving and post-calving body condition score (BCS) change were more positive with supplementation ($P < 0.01$), while monensin addition to the supplements benefited pre-calving ($P = 0.02$) and post-calving ($P = 0.02$) BCS change a greater amount with the CU supplement compared with the DDGS supplement. Monensin addition, irrespective of supplement type, reduced forage intake while maintaining performance of beef cattle consuming low-quality forage.

Key words: cattle, cool-season, forage, ionophore, monensin, supplementation

INTRODUCTION

Beef cattle producers have taken advantage of ionophores, such as monensin and lasalocid, since the 1970's (Perry et al., 1976; Turner et al., 1980). The principle advantages associated with incorporating ionophores into beef cattle diets are improved feed efficiency and amelioration of digestive upsets. In addition, ionophores have proven useful in helping control certain health disorders such as liver abscesses and coccidiosis. As a result, ionophores improve the cost of production in the growing/feedlot by almost $12/head, with approximately 93% of all feedlots currently using ionophores (Lawrence and Ibarburu, 2007). Another benefit of using ionophores in ruminant diets is a reduction in greenhouse gas emissions. Research has noted that methane production by cattle (21 times more powerful than carbon dioxide as a greenhouse gas) can be decreased almost 40% when monensin is included in the diet (Neto et al., 2009). It should be noted that the vast majority of the aforementioned research was conducted with growing cattle consuming high-concentrate diets or high quality pasture (Bretschneider et al., 2008). In contrast, there is little data available related to feeding ionophores to mature cattle consuming low-quality, forage-based diets (Bretschneider et al., 2008). Also, there is a paucity of research evaluating the effect of supplement type and ionophore addition on beef cattle consuming poor-quality forages.

The majority of research documenting improved feed efficiency when ionophores are provided to ruminants consuming low-quality forage has noted comparable animal performance when provided 10% less forage compared with animals not receiving ionophores (Bretschneider et al., 2008). Nevertheless, Lemenager et al. (1978) suggested that forage intake was reduced from 15 to 20% by beef cows grazing winter range in Oklahoma and supplemented with monensin compared with those not receiving monensin. If cattle producers that use low-quality forages for a significant period of the year can reduce the quantity of forage utilized while maintaining or improving animal performance, simply by supplementing with an ionophore, they can reduce required winter feed resources, decrease winter feed costs, and potentially reduce the environmental impact of their operation. We hypothesize that providing supplemental monensin to beef cattle will decrease intake of low-quality forage while maintaining performance; thereby improving feed efficiency, energy status, and ruminal fermentation compared with no monensin. In addition, we hypothesize that the beneficial effects of monensin will be independent of supplement type.

MATERIALS AND METHODS

Experiment 1. Influence of Supplement type and Monensin Addition on Forage Intake and Digestibility in Steers

Five ruminally cannulated Angus x Hereford steers (450 ± 25 kg) were used in an incomplete 5 x 4 Latin square and housed in individual pens within an enclosed barn with continuous lighting. Treatments included a non-supplemented control (CON), approximately 30% CP supplements consisting of either corn and urea (CU; 0.29% BW), CU + monensin (200 mg/day; CU+M), dried distiller's grains (DDGS; 0.27% BW), or DDGS + monensin (200 mg/day; DDGS+M). All supplemented treatments were formulated to be provide similar caloric and nitrogen intakes.

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Supplements and a mineral-salt mix (Cattleman's Choice; Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 6000 mg/kg Zn, 3200 mg/kg Cu, 65 mg/kg I, 900 mg/kg Mn, 140 mg/kg Se, 136 IU/g of vitamin A, 13 IU/g of vitamin D3, and 0.05 IU/g of vitamin E were placed directly into the rumen via ruminal cannula daily. Steers had continuous access to fresh water and chopped fine fescue grass seed straw (4.5% CP).

The 4 experimental periods were 28 d each with 20 d of diet adaptation and 8 d of sampling. Forage intake was measured d 21 through d 26 and blood samples were collected into commercial blood collection tubes via coccygeal venipuncture 4 h after feeding on d 23 through d 28. Also, on d 28 approximately 100 mL of ruminal fluid was collected by suction strainer immediately prior to hay feeding and at 2, 4, 6, 8, 12, 18, and 24 h after feeding. Ruminal fluid pH was measured immediately after collection. Five mL was acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (−20°C) for subsequent analysis of VFA.

Plasma glucose and plasma urea N (PUN) concentrations were determined using quantitative colorimetric kits (number G7521 and B7551, respectively; Pointe Scientific, Inc., Canton, MI). Concentration of IGF-I was determined as described by Moriel et al. (2012). Insulin concentration was determined using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostics, Los Angeles, CA) previously validated for bovine samples (Moriel et al., 2008)

Intake and digestibility data were analyzed as a 5 x 4 incomplete Latin square with the MIXED procedure of SAS. The model included period and treatment and steer was used as the random variable. Contrasts used to partition specific treatment effects consisted of: 1) supplemented vs non-supplemented; 2) Monensin addition; 3) supplement type; and 4) the monensin addition by supplement type interaction.

Ruminal pH and VFA were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included period, treatment, hour and treatment x hour. Steer was used as the RANDOM statement to specify variation and steer(period) was used as the subject. The specific term for the repeated statement was hour. Autoregression (AR1) was determined to be the most appropriate covariance structure based on the Akaike information criterion. The same contrasts as previously noted were used to partition specific treatment effects.

Blood samples were analyzed using the REPEATED statement with the MIXED procedure of SAS. The model included period, treatment, day and treatment x day. Steer was used as the random variable and steer(period) was used as the subject. The specific term for the repeated statement was day. Autoregression (AR1) was determined to be the most appropriate covariance structure based on the Akaike information criterion. The same contrasts as previously noted were used to partition specific treatment effects. If no treatment x time interactions were detected ($P > 0.05$), overall treatment means were compared.

Experiment 2. Influence of Supplement type and Monensin Addition on Cow Performance

Eighty late gestation (approximately 190 d pregnant) Angus x Hereford cows (532 ± 79 kg) were stratified by age, BCS, and BW. Cows were then randomly assigned to 1 of 5 treatments. The same treatments as described in Exp. 1 were used. Water and a mineral-salt mix was available free choice (same composition as previously described; Cattleman's Choice; Performix Nutrition Systems, Nampa, ID). Cows were provided ad libitum access to low-quality (approximately 4.3% CP) fine fescue grass seed straw.

Cow BW and BCS were measured every 14 d until calving and within 24 h post-calving. Calf BW was also obtained within 24 h post-calving. Blood samples were via jugular venipuncture at trial onset and within 24 h post-calving. Straw, corn, and DDGS samples were collected weekly and analyzed for CP, OM, NDF, and ADF. Cow BW and BCS were measured every 14 d until calving and within 24 h post-calving. Calf BW was also obtained within 24 h post-calving. Blood samples were collected into 2 commercial 10-mL blood collection tubes (1 containing 0.1 mL of a 15% EDTA solution for plasma harvest and 1 vacutainer for serum harvest) via jugular venipuncture at trial onset and within 24 h post-calving. Plasma glucose, PUN, Insulin, and IGF-1 concentrations were determined as described in Exp. 1. In addition, serum NEFA concentration was determined as described by Pescara et al. (2010).

Cow performance data was analyzed using the MIXED procedure of SAS. The model included treatment and cow(pen) and pen(treatment) were used as the random variables. Blood samples were analyzed using the REPEATED statement with the MIXED procedure of SAS. Model included treatment, day, and treatment × day with cow(pen × treatment) used as the random variable. Initial blood values were used as a covariate. Day was used as the repeated variable; the subject was cow(pen). The same contrasts as previously described were used to partition specific treatment effects.

RESULTS AND DISCUSSION

Exp. 1 Forage intake, digestibility and ruminal fermentation characteristics in steers

Intake. Forage intake was not altered by supplementation (Table 1; $P > 0.05$); however, DDGS increased forage intake 6% compared with the CU supplement ($P = 0.03$). Also, monensin addition to supplements reduced forage intake by greater than 5% ($P = 0.04$). Total dry matter intake was increased with supplementation ($P < 0.01$), but decreased with monensin addition ($P = 0.04$).

Ruminal Fermentation Ruminal pH was not altered with supplementation ($P = 0.58$); however, we did note that monensin addition to the DDGS supplement increased average ruminal pH 0.3 units compared with only 0.1 units with the CU ($P < 0.01$; Table 1).

Total VFA increased with monensin addition to CU but decreased when added to DDGS (Table 1; $P = 0.01$). Also, molar proportions of propionate, iso-butyrate, and isovalerate increased with supplementation ($P \leq 0.05$) and with monensin ($P < 0.01$) while acetate and acetate:propionate decreased with supplementation ($P <$

0.01) and acetate, acetate:propionate, and valerate decreased with monensin ($P < 0.01$). Also, the molar proportion of iso-valerate was greater with CU compared with DDGS ($P < 0.01$).

Blood Variables. Insulin was not affected by treatments ($P \geq 0.019$) but glucose was greater with the CU compared with the DDGS supplements (63 vs 57 ng/mL; $P = 0.01$), probably due to the greater starch content of the corn compared with distiller's grains. Protein supplementation has been shown to increase PUN and IGF-I in beef cattle. Our data supports this as plasma IGF-I and BUN concentrations were increased with supplementation ($P < 0.01$; Table 1). Furthermore, IGF-I has been shown to increase with greater DMI (Rausch et al., 2002), suggesting that our increase in IGF-I with supplementation may have been due to greater energy and DM intake resulting from supplementation. Also, due to the greater ruminal degradability of protein in the CU supplement, BUN was almost 100% greater with the CU compared with DDGS ($P < 0.01$; 24 vs 13 mg/dL).

Exp.2 Influence of Supplement Composition on Cow Performance

Protein supplementation of beef cows consuming low-quality forage typically improves weight and BCS change compared with not providing a supplement (Bohnert et al., 2002). This was observed in the current study for pre- and post-calving weight and BCS change ($P < 0.01$; Table 1). Also, we observed an increase in pre- and post-calving weight change due to supplement type, with DDGS increasing weight gain compared with CU supplementation. Interestingly, monensin supplementation improved pre-calving cow BCS 0.4 units with the CU supplement compared with a loss of 0.2 units with the DDGS supplement ($P = 0.02$). Similarly, post-calving BCS change was improved 0.4 units with monensin addition to CU compared with a 0.1 increase with DDGS ($P = 0.02$). This suggests monensin was more advantageous when incorporated into the CU supplement compared with DDGS.

As with the steers in Exp. 1, plasma insulin was not altered by the treatment regime ($P \geq 0.32$; Table 2) but glucose was increased ($P = 0.05$) with the CU supplements compared with DDGS. Plasma IGF-1, an indicator of overall nutritional status, increased with supplementation ($P < 0.01$) but was not affected by supplement type or monensin addition ($P \geq 0.12$). Also, as noted in Exp. 1, BUN was greater with supplementation compared with the non-supplemented control ($P < 0.01$) and for the CU compared with DDGS supplements ($P = 0.02$).

IMPLICATIONS

Inclusion of monensin into starch- or fiber-based CP supplements for beef cattle consuming low-quality, cool season forages, can be a management strategy to reduce forage intake while maintaining performance. Also, based on cow BCS change, starch-based supplements may benefit more from monensin addition than non-starch-based supplements.

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Table 1. Effects of supplement type and monensin on intake, ruminal fermentation, performance, and blood variables in ruminants consuming low-quality, cool-season forage

	Treatment[a]						Contrasts[c], P =			
	Con	CU	CU+M	DDGS	DDGS+M	SEM[b]	Con vs Supp	M	Supp Type	M vs Type
Experiment 1										
Intake, % of BW										
Forage	1.41	1.51	1.39	1.56	1.52	0.52	0.06	0.04	0.03	0.25
Supplement	0.0	0.29	0.29	0.27	0.27					
Total	1.41	1.80	1.68	1.83	1.79	0.52	<0.01	0.04	0.07	0.25
Ruminal pH	6.75	6.68	6.76	6.59	6.88	0.048	0.58	<0.01	0.65	<0.01
Ruminal Total VFA, mMol	108.2	120.4	125.7	128.6	104.7	5.80	0.08	0.11	0.26	0.01
Acetate, mol/100 mol	58.4	56.8	51.4	55.5	52.9	1.06	<0.01	<0.01	0.87	0.09
Propionate, mol/100 mol	18.5	17.9	20.9	18.9	20.6	0.48	0.05	<0.01	0.46	0.18
Iso-butyrate, mol/100 mol	2.3	2.6	3.2	2.4	3.5	0.17	<0.01	<0.01	0.52	0.11
Butyrate, mol/100 mol	13.5	13.6	12.8	14.7	12.7	0.88	0.99	0.12	0.61	0.49
Iso-valerate, mol/100 mol	3.4	5.0	8.4	3.7	6.3	0.41	<0.01	<0.01	<0.01	0.23
Valerate, mol/100 mol	4.0	4.3	3.6	4.5	3.8	0.31	0.96	<0.01	0.32	0.93
Acetate:Propionate	3.2	3.2	2.5	3.0	2.6	0.12	<0.01	<0.01	0.63	0.11
Insulin, ng/mL	4.07	3.60	3.75	3.29	3.26	0.89	0.19	0.88	0.31	0.82
Glucose, mg/dL	58.7	63.9	62.4	59.8	54.3	2.41	0.57	0.12	0.01	0.36
IGF-I, ng/mL	107	165	175	174	178	17.7	<0.01	0.27	0.31	0.66
Plasma urea N, mg/dL	9.6	24.0	23.6	12.4	14.5	2.02	<0.01	0.47	<0.01	0.30
Experiment 2										
Initial Wt., kg	521	524	547	533	544	34.1	0.68	0.62	0.93	0.86
Initial BCS	4.8	5.2	4.8	4.9	4.8	0.16	0.38	0.17	0.49	0.22
Weight change, kg										
Precalving	4	45	57	84	74	9.5	<0.01	0.89	<0.01	0.26
Postcalving	-42	4	6	36	23	11.2	<0.01	0.60	0.04	0.50
BCS change										
Precalving	-0.8	-0.3	0.1	0.2	0.0	0.14	<0.01	0.41	0.10	0.02
Postcalving	-0.9	-0.6	-0.2	-0.1	0.0	0.17	<0.01	0.08	0.06	0.02
Calf Birth Wt., kg	35.6	36.9	38.4	41.3	37.6	1.81	0.15	0.53	0.34	0.16
Insulin, ng/mL	6.2	4.0	5.5	3.8	4.1	1.65	0.32	0.57	0.61	0.73
Glucose, mg/dL	77.8	80.3	85.8	76.6	74.5	3.95	0.71	0.66	0.05	0.32
IGF-I, ng/mL	25.9	44.8	47.4	53.3	60.2	6.51	<0.01	0.46	0.12	0.74
Plasma urea N, mg/dL	10.9	20.3	19.6	13.6	18.0	1.64	<0.01	0.27	0.02	0.13
NEFA, mEq/L	0.51	0.53	0.52	0.59	0.60	0.060	0.45	0.96	0.28	0.81

a Con = control; CU = corn/urea; CU+M = CU + 200 mg of monensin; DDGS = dried distillers grains with solubles; DDGS+M = DDGS + 200 mg of monensin.

b n = 5

c Con vs Supp = control vs supplemented treatments; M = effect of monensin addition; Supp Type = effect of supplement type; M vs Type = Interaction of monensin addition and supplement type.

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Immunodetection of the Cry toxin in leaves of transgenic maize hybrids*

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ABSTRACT: This assay aimed to evaluate the Cry toxins concentration in the last completely expanded leaf at 7, 14, 28, 35, 70, 84 and 96 days after planting of five corn hybrids. The evaluated hybrids were Syngenta Impact TL TG, Monsanto DKB 390 VT PRO II, Monsanto AG 8088 PRO II, Biomatrix 2B655 Herculex from Dow and Syngenta 7205 Viptera. To analyze the toxins concentrations antibodies were produced and a PTA-ELISA developed. The positive controls used to produce the calibration curves were Sigma-Aldrich BF412b (Cry1Ab), Sigma-Aldrich BF418b (Cry1F) and Sigma-Aldrich BF423b (Vip3Aa20). The experimental design was randomized blocks in a factorial arrangement 5 (hybrid) x 7 (samples) with five repetitions. There were a significant interaction between hybrid and sampling period. The mean concentrations of the Cry toxins in the hybrids Herculex, AG 8088 PRO II, DKB 390 VT PRO II, Viptera and Impacto were 7.58, 13.05, 16.81, 22.67, and 34.05 µg/g of fresh tissue, respectively. The mean concentration of Cry toxins were 16.77, 15.16, 18.46, 22.87, 22.85, 20.62 and 15.98 µg/g of fresh tissue at 7, 14, 28, 35, 70, 84 and 96 days after planting. Higher concentration of Cry toxin occurred between 35 and 70 days after planting and the hybrids with the higher concentrations were Viptera and Impacto with concentrations of 34.6 and 39.6 µg/g of fresh tissue, respectively. This information can integrate selection criteria of hybrids for cultivation in different regions according to the level of infestation of pests and resistance thereof to different Cry toxins concentrations.

Key words: Corn, Cry toxin, OGM, PTA ELISA

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INTRODUCTION

The competition provided by pest resistance made transgenic corn hybrids expressing Cry toxins occupy most of the arable land in short time. The whole phenomenon of Cry toxin production variation by plants are not fully understood and the level of exposure in the field remains unknown. To monitor the environmental exposure of Cry toxins and pest resistance to it, is necessary to quantify it in different hybrids over the cultures development. However, the analysis commonly performed limit to state whether the plant is or not GMOs. The objective of this study was to evaluate the concentrations of Cry toxins in five transgenic hybrids leaves harvested at 7, 14, 28, 35, 70, 84 and 96 days after planting.

MATERIALS AND METHODS

The parameters studied were the toxin concentrations in the leaves throughout the development of hybrids Syngenta Impact TL TG, Monsanto DKB 390 VT PRO II, Monsanto AG 8088 PRO II, Biomatrix 2B655 Herculex Dow and Syngenta Viptera 7205.

To obtain rabbit antiserum against Cry toxins, a preparation of 0.5 g of DKB VT 390 Pro II leaves lyophilized diluted in PBS, was used as source of antigen (Cry toxin). The Cry-enriched preparation (antigen) was obtained from aqueous extract of DKB VT 390 Pro II leaves using ultradiaflitration system (Millipore, cut off between 50 and 100 KDa). The Cry antigen was conjugated with a protein carrier, bovine serum albumin (Cry-BSA), using the carbodiimide method. Hyper-immunization of a rabbits with CryBSA were executed by classic protocol (Harlow & Lane, 1988). All rabbits experiments were performed under approved conditions in accordance Institutional Animal Care and User Committee approved protocols from APTA-SAA (CEEA/IZ 0047).

It was used an immunoassay type PTA-ELISA (Plate-Trapped Antigen - Enzyme Linked Immuno Sorbent Assay). The set of samples per plate was composed of seven collection periods, positive control (corresponding to Cry toxin expressed by the hybrid in question) and conventional isogenic. As positive controls were used products from Sigma-Aldrich BF412b (Cry1Ab), Bt11 event, used for evaluation of hybrids Syngenta Impact TL TG, Monsanto DKB 390 VT PRO II and Monsanto AG 8088 PRO II, Sigma-Aldrich BF418b (Cry1F) to evaluate the hybrid Biomatrix 2B655 Herculex Dow and Sigma-Aldrich BF423b (Vip3Aa20), MIR 604 event, used to evaluate the hybrid Syngenta Viptera 7205.

The values in% GMO were converted into ng/g according to Volpe et al (2006) (y = 2.06 x + 0.01, R2 = 0.97) and thus, via a calibration curve were known the amounts of Cry proteins in the samples in ng. Then it was considered the amount of sample and dilution to calculate the amount of the Cry proteins in 1g, yielding values in ng/g. The values were multiplied by 1000 through the drive µg/g. Subsequently, the dry matter content of the sample was considered standardizing the units in µg/g of fresh tissue.

The evaluations were in the last fully expanded leaf whole with 7, 14, 28, 35, 70, 84 and 96 days after planting. Samples were frozen, lyophilized, and grinded into particles below 40 mesh. The extraction of the protein was performed on 100 mg of lyophilized leaf macerated in liquid nitrogen, suspended in 1 mL of methanol (80%) and diluted in 4 mL of Sigma PBS (Phosphate Buffer Saline) prepared with distilled water. The samples were transferred to Eppendorf tubes, shaken for 1 minute by vortexing and then centrifuged for 5 minutes at 6,500 rpm. The supernatant (100 µl) was added in duplicate for sensitization in titration wells. Were used microtiter plates 96-well flat bottom (Corning High Binding, USA), the upper part of the plate being covered with parafilm to avoid evaporation, and carried the incubation with gentle movement, at 37ºC for 1h. Then they were added 150 µL of 3% fish gelatin lock (Difco, USA) dissolved in PBS and incubating held at 37ºC for 1h. Thereafter, the plates were hitted against paper towels to remove all liquid from the wells and then was added 100 µl of serum, diluted in PBS (1:1000). Then the plate was sealed with wrap to prevent evaporation and incubated at 37°C for 1h. After the initial steps the plates were washed three times with 150 µl of wash solution (PBS diluted in 1 liter of distilled water plus 0.05% Tween 20%). Were added sequentially 100 µL in each well of anti-rabbit conjugate labeled with alkaline phosphatase, diluted in PBS (1: 10,000), the plate was sealed with film and incubated at 37 ° C for 1h. After incubating the antibody conjugated it was done further washing and beats as described above and proceeded disclosure for 15 minutes in the dark by adding 100 µl of TMB solution (tetramethylbenzidine). After the development period was added 50 µl of sulfuric acid as stop solution ($H_2SO_4$2N = 5.55 ml acid to 100 mL H_20) in all wells. Subsequently, the plate remained uncovered at room temperature and reading was carried out at 450 nm. The readings of optical density (OD) of the samples were compared with cutting of the reference readings, discounting the read values of the negative controls (samples of conventional and white isogenic plants) and recorded on regression equations obtained by positive controls, to estimate the concentration of the Cry toxin in the sample.

RESULTS AND DISCUSSION

Given that the points for the curve to be obtained in the same assay and although the studied hybrids contain *Bacillus thuringiensis* genes that express different toxins, specific regression equations were obtained for each case. Table 1 equations are obtained for the quantification of Cry toxins sheets.

There was a significant interaction of Cry toxin concentration in the sheet between hybrids and collection periods (Table 2). The influence of plant age on the concentration of toxins in the sheet differed among the hybrids, occurring higher concentrations from 35 to 70 days after planting.

Several factors may influence the concentration of Cry1Ab protein in plant tissues by altering the production of Cry1Ab protein by the plant and hence its availability for insects and environment. Indeed, the physiological state of the plant, the weather, the seasons, the interactions between genotype and environment (NGUYEN and JELH, 2007), nitrogen fertilization (BRUNS and ABEL, 2003), soil quality and pesticides applied (GRIFFITHS et al. 2006) are factors that can change the Cry1Ab protein production by the plant.

The hybrids with the highest concentrations were Viptera and Impacto, probably more efficients in controlling pests. The information generated can integrate a criteria in chosen hybrids for cultivation in different regions according to the level of infestation by pests and resistance thereof to different concentrations of Cry toxins.

The intake of non-lethal doses of toxins by insects may represent a contributing factor to the resistance, increasing the tolerance of pests upon ingestion of leaves containing Cry toxin. This occurrence would preclude control of pests without successive sprayings of chemical insecticides, greater purpose of the use of GMOs studied. Knowing the concentration of toxins in hybrid, allows to estimate the tolerance of insects targets the toxin, ie, what is the survival of the insects after ingestion of a certain amount of toxin. Thus, comparing the concentration of Cry toxins between hybrids, as well as allowing the selection of hybrid to be cultivated in accordance with the infestation history for pests in the region, can guide the use of alternation thereof, with the objective of slowing tolerance of pests Cry toxins and extend the efficiency of hybrid control of the same, without the use of insecticides.

IMPLICATIONS

There are variations in the concentrations of Cry toxins of different hybrids of corn, and throughout the cycle of these cultures should be considered in choosing the hybrid to be cultivated.

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Table 1 - Regression equations by Sigma-Aldrich patterns and their hybrid for analysis of expression of Cry toxins in leaves

Hybrids	Protein	Regression equations	R^2	Pr	CV	MSE
DKB390	Cry1A105 +Cry2Ab2	Y=-0.1264+(0.65091*X)	0.99	0,001	9.13	0.060
AG8088	Cry1A105 +Cry2Ab2	Y=-0.1264+(0.65091*X)	0.99	0.001	9.13	0.060
Herculex	Cry1F	Y=-0.020765+(0.06284*X)+(0.732801*X^2)	0.99	0.003	4.91	0.003
Impacto	Cry1Ab	Y=-0.1264+(0.65091*X)	0.99	0.001	9.13	0.060
Viptera	Vip3Aa20	Y=-0.117928+(0.62964*X)+(0.005866*X^2)	0.99	0.053	12.87	0.085

Table 2 – Cry toxin expression in µg/g of fresh tissue of the last completely expanded leaf of different hybrids

Days	DKB390	AG8088	Herculex	Impacto	Viptera	CV	MSE	Pr
7	17.49^{Bb}	8.21^{Ccd}	2.52^{Cd}	34.76^{Abc}	20.86^{Bbc}	21.48	3.93	<0.0001
14	5.81^{Cd}	8.74^{BCc}	2.86^{Cd}	43.13^{Aa}	15.28^{Bc}	25.55	3.77	<0.0001
28	17.95^{ABCb}	14.29^{BCb}	7.29^{Cc}	31.03^{Ac}	21.75^{ABbc}	37.07	7.41	0.0006
35	21.92^{Ba}	22.85^{ABa}	9.82^{Bb}	39.61^{Aab}	20.64^{Bbc}	39.31	8.59	0.0008
70	22.11^{ABa}	15.53^{Bb}	11.23^{Bab}	30.72^{Ac}	34.65^{Aa}	31.89	7.38	0.0004
84	19.93^{Bab}	16.29^{Bb}	6.91^{Cc}	30.60^{Ac}	29.36^{Aab}	20.81	4.34	<0.0001
96	12.49^{c}	5.41^{d}	12.44^{a}	33.41^{bc}	16.12^{c}	42.16	7.30	<0.0001
CV	35.86	42.30	47.71	18.52	40.10			
MSE	6.16	5.71	3.50	6.39	9.00			
Pr	0.005	0.006	<0.001	0.018	0.025			

Capital letters in line and lower in column indicate significant difference ($p<0.05$)

GRADUATE STUDENT
PAPER COMPETITION

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Effects of dried distiller's grains and lasalocid on feedlot lamb growth, carcass traits, nutrient digestibility, ruminal fluid volatile fatty acid concentrations, and ruminal hydrogen sulfide concentration

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ABSTRACT: Our hypothesis was increasing the inclusion level of dried distiller's grains with solubles (**DDG**) to feedlot lambs would increase growth, while the inclusion of lasalocid (**LAS**; Bovatec, Alpharma, LLC, Bridgewater, NJ) would increase ADG and G:F, while not affecting digestibility, ruminal VFA concentration, and pH in the rumen fluid. Furthermore, we hypothesized that rations including LAS and higher levels of DDG would cause increased ruminal hydrogen sulfide gas (**H_2S**) concentrations. To test this hypothesis, two hundred forty crossbred (Suffolk × Rambouillet) lambs (31.9 ± 5.87 kg BW; approximately 90 d of age) were allocated to 6 treatments in a completely random design with a 3 x 2 factorial arrangement of treatments. Lambs were placed into 24 feedlot pens (4 pens/treatment; 10 lambs/pen) for a 111 d finishing study. Main effects included concentration of DDG (0, 15, or 30% DM basis) and inclusion of LAS (0 or 20 g/ton LAS) resulting in treatments of 1) 0% DDG without LAS (**0DDG-NL**), 2) 0% DDG with LAS (**0DDG-L**), 3) 15% DDG without LAS (**15DDG-NL**), 4) 15% DDG with LAS (**15DDG-L**), 5) 30% DDG without LAS (**30DDG-NL**), and 6) 30% DDG with LAS (**30DDG-L**). Two-day weights were taken at the beginning and end of the trial. Two hundred eighteen lambs (63.7 ± 8.78 kg) were harvested on d 112 at a commercial abattoir and carcass data collected after a 24 h chill. The inclusion of LAS increased ($P \leq 0.02$) final BW, ADG, G:F, and HCW. As DDG in the ration increased to 30%, DMI decreased linearly ($P = 0.03$) while G:F increased linearly ($P = 0.03$). A second study was conducted utilizing the same treatments to evaluate N and S balance, ruminal VFA and H_2S concentration, and ruminal pH in 24 crossbred wethers (Suffolk × Rambouillet; 41.2 ± 12.23 kg BW). Daily urinary sulfur excretion and H_2S production were linearly increased ($P < 0.001$) as DDG increased in the ration. Total ruminal VFA concentration linearly decreased ($P = 0.002$) as DDG increased in the ration. The inclusion of LAS increased ($P = 0.02$) ruminal pH. The results confirm our hypothesis that LAS increased overall growth and increasing DDG increased ruminal H_2S concentration, however, DDG inclusion did not increase growth. Additionally, we reject the hypothesis that the combined effects of LAS and DDG would have no effect on rumen pH and VFA concentrations.
Key words: dried distiller's grains with solubles, feedlot, ionophores, lambs, sulfur, volatile fatty acids

INTRODUCTION

Ethanol production in the United States continues to increase (Renewable Fuels Association, 2016). Dried distiller's grains with solubles (**DDG**) is an affordable byproduct of ethanol production and also serves as an excellent supplementary feed for livestock as it is high in crude fat and RUP. However, many producers are apprehensive about feeding DDG to feedlot lambs above 20% of the ration for fear of S toxicity. Multiple research projects have been performed assessing the feeding of DDG to feedlot lambs (Huls et al., 2006; Neville et al., 2010; Schauer et al., 2008), with no negative effects on performance or morbidity observed, even at inclusion levels of 60% of the diet. In fact, Crane et al. (2015) observed that feeding rations containing 45% DDG tended to increase feedlot performance in growing rams. However, none of the previous DDG research in lambs has evaluated the inclusion of lasalocid (**LAS**; Bovatec, Alpharma, LLC, Bridgewater, NJ) to potentially further increase feedlot performance through the benefits of an ionophore (increased feed efficiency; Funk et al., 1986; Crane et al., 2014). By inhibiting hydrogen- and ammonia-producing bacteria, LAS decreases the acetate:propionate ratio and increases feed efficiency (Bartley et al., 1979). Kung et al. (2000) determined hydrogen sulfide (**H_2S**) production may increase when ruminants are fed ionophores, such as LAS. Therefore, we hypothesized that increasing the inclusion level of DDG to feedlot lambs would increase growth, while the inclusion of LAS would increase ADG and G:F, while not affecting digestibility, VFA concentrations, and pH in the ruminal fluid. Furthermore, we hypothesized that the rations including LAS and higher levels of DDG would cause increased ruminal H_2S concentrations. Our objectives were to evaluate the interaction of DDG and LAS on feedlot lamb performance and ruminal digestibility parameters.

MATERIALS AND METHODS

All procedures were approved by the Animal Care and Use Committee at North Dakota State University.

Feedlot Study

Animals and Diets. At 2 wk of age, tails were docked, males were castrated, and all lambs were vaccinated (**CD-T**;

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Bar Vac CD/T; Boehringer Ingelheim, Ridgefield, CT). Lambs were adapted to an 80% corn and 20% commercial market lamb pellet diet (DM basis; Table 1) from a 100% creep meal diet following weaning at approximately 60 d of age. Lambs were vaccinated with CD-T again at 60 d of age and d -1 of the study. In May 2016, two hundred forty crossbred (Suffolk × Rambouillet) lambs were stratified by BW (31.9 ± 5.87 kg; approximately 90 d of age) and sex (105 wethers and 135 ewes) and randomly assigned to 1 of 16 outdoor pens (10 lambs/pen). No treatment related morbidity or mortality was observed. Pens were assigned randomly to 1 of 6 treatments, with pen serving as the experimental unit (n = 4 pens/treatment). Diets were based on an 80% corn and 20% market lamb meal (**MLM**) diet, which included LAS for respective treatments, and were balanced to be isonitrogenous and equal to or greater than the CP and NE requirements (NRC, 2007) for a 40 kg lamb gaining 300 g/d. Rations were formulated to have a minimum Ca: P ratio of 2:1. Rations were ground through a 1.27 cm screen (Gehl Mix-All, Model 170, Gehl, West Bend, WI), mixed, and offered ad libitum via bulk feeders (48.6 cm bunk space/lamb). Lambs had continuous access to clean, fresh water and shade. Feeders were checked daily and cleaned of contaminated feed. Lambs were observed daily to monitor health and treated when necessary. Main effects included dietary concentration of DDG (0, 15, or 30% DM basis) and inclusion of LAS (0 or 20 g/ton LAS) resulting in treatments of 1) 0% DDG without LAS (**0DDG-NL)**, 2) 0% DDG with LAS (**0DDG-L**, 3) 15% DDG without LAS (**15DDG-NL**), 4) 15% DDG with LAS (**15DDG-L**), 5) 30% DDG without LAS (**30DDG-NL**), and 6) 30% DDG with LAS (**30DDG-L**).

Data Collection Procedures. Lambs were weighed on two consecutive d at the initiation (d -1 and 0) and end (d 110 and 111) of the trial; single day weights were taken on d 28, 54, and 84. Feed ingredient and ration grab-samples (approximately 0.2 kg) were collected from the bulk feeders at the beginning of each period and dried at 55°C for 48 h to determine DM and ration nutrient composition. Dried samples were ground to pass a 2-mm screen. Samples were analyzed for DM, ash (AOAC Int., 2010), N (AOAC Int., 2010) using a Kjeltec Auto 1030 Analyzer (Tecato AB, Höganäs, Sweden), mineral content including S (AOAC Int., 2010), NDF (Van Soest et al., 1991) as modified by Ankom Technology (Fairport, NY) using an Ankom 200 Fiber Analyzer without sodium sulfite, with amylase, and without ash corrections as sequentials, and ADF (Goering and Van Soest, 1970). On d 112 of the trial, lambs (218 hd; 64.8 ± 7.99 kg BW) were harvested at Mountain States Rosen Company (Greeley, CO). Trained personnel collected carcass data after a 24-h chill (temperature < 2°C and humidity near 100%). Carcass data collected included HCW, fat depth, loin eye area (**LEA**), and body wall thickness (at the 12th rib), conformation score, flank streaking, lean maturity, yield grade, and % boneless closely trimmed retail cuts (**BCTRC;** Savell and Smith, 2000). Conformation score was scored on a scale of 100 to 1500 (100 = cull; 1500 = prime). All lambs were assigned bone maturity of A with varying lean maturity scored on a scale of 0 to 100 (0 = very fine; 100 = fine). Flank streaking was assigned with scores of 100 to 199 = Practically Devoid, 200 to 299 = Traces, 300 to 399 = Slight, 400 to 499 = Small, and 500 to 599 = Modest.

Digestibility Study

Animals and Diets. Twenty-four crossbred (Suffolk x Rambouillet) wethers (41.2 ± 12.23 kg BW; approximate age = 90 d) were used in completely random design with a 3 x 2 factorial arrangement of treatments as described in the feedlot study. Wethers were weighed on d 0 and 1, stratified by weight, and allotted randomly to treatments (*n* = 4 wethers/treatment). Lambs were assigned randomly to individual metabolism crates on d 1. Wethers were housed in an enclosed room with lighting from approximately 0730 to 2000 h. Lambs were adapted to diets (Table 1) and processed as outlined in the previous study, but lambs were also given an injection of vitamins A, D and E on d 1 of the trial. Rations were provided daily at 0830 h at 130% of the average daily intake for the previous 5 d. Feed refusals from the previous day were determined before feeding. Water troughs were cleaned and refilled daily after feeding.

Data Collection Procedures. The experimental period was 23 d. Dry matter intake was determined on d 14 to 20. Additionally, samples of DDG, corn, and MLM were collected on d 14 to 20 and dried at 55°C for 48 h to determine DM. Orts (weigh backs) were collected on d 15 to 21 and dried at 55°C for 48 h. Wethers were fitted with fecal collection bags on d 13. Total fecal and urine output were collected on d 17 to 23. A subsample of each daily fecal sample (7.5% of total, wet basis) was dried at 55°C for 96 h for calculation of fecal DM. Urine was collected via stainless steel funnel beneath the lamb, with total urine output collected. Sufficient 6 *N* HCL (100 mL) was added daily to urinals to maintain urine pH < 3. Total daily urine output was recorded and urine was composited daily by wether (10% of total; wet basis) and stored at 4°C. Approximately 288 g of urine were collected from each urine subsample and stored at 4°C. On d 15 to 21, 10 mL of blood were collected via jugular venipuncture 4 h after feeding using vacutainers (VWR International). Blood was cooled at 4°C for 2 h and centrifuged (3,640 × *g*, 15 degrees C, 20 min), and serum was harvested and stored (-20°C).

Dried fecal samples were ground to pass a 2-mm screen and composited by lamb. Daily samples of corn, MLM and ration were composited for the collection period, and orts were composited by lamb on an equal weight basis (20%; as-fed basis). Feed, orts, and fecal samples were analyzed for DM, ash, NDF, and ADF as described previously. Feed, orts, fecal, and urine samples were analyzed for N and S as described previously. Concentration of N and S in feed, orts, fecal, and urine samples was used to calculate daily N and S intake and excretion from feed, ort, feces, and urine weights. Nitrogen excretion (fecal N + urinary N) was subtracted from N intake (feed N – ort N) to calculate N balance (g N/kg BW basis). Sulfur excretion and balance were also determined using the same calculations. Serum samples were analyzed for urea-N using the Sigma Diagnostics Procedure 640

(Sigma Chemical Co., St. Louis, MO) and an ultraviolet-visible spectroscopy spectrophotometer (DU 800 Spectrophotometer, Beckman Coulter, Brea, CA). Serum-S concentrations were also determined by Midwest Laboratories Inc., (Omaha, NE) using Inductively Coupled Plasma.

Ruminal gas cap sampling was conducted on the same twenty-four lambs in the N and S balance study on d 15 and 23 utilizing procedures outlined by Neville et al. (2010). Gas samples were collected 4 h after feed was offered. Ruminal fluid was collected via rumenocentosis at the same time ruminal gas cap samples were collected for determination of ruminal fluid VFA concentrations and pH. On both sampling days (15 and 23), duplicate measurements were taken from each lamb, and the mean of the 2 samples was used for calculations.

Statistical Analysis

Data were analyzed as a completely randomized design using the Mixed procedure of SAS (v. 9.3; SAS Inst. Inc., Cary, NY), with pen serving as experimental unit in the feedlot study and lamb serving as experimental unit in the digestibility study. Serum S concentrations were only analyzed on d 18 of the digestibility trial, and were analyzed using the MIXED procedure of SAS. Repeated measures were used for the analyses of serum urea-N, individual and total VFAs, and H_2S concentrations, as well as rumen fluid pH. Following protection with an overall F-test for treatment ($P < 0.05$), if a DDG x LAS interaction occurred ($P < 0.05$) means were separated using the LSMEANS procedure of SAS and P-values ≤ 0.05 were considered different. Linear and quadratic contrasts were used to evaluate the effects of increasing inclusion of DDG in the ration.

RESULTS AND DISCUSSION

Feedlot Performance and Carcass Characteristics

There were no interactions ($P \geq 0.24$) of DDG and LAS inclusion for final BW, ADG, DMI, or G:F (Table 2). The inclusion of LAS increased ($P \leq 0.02$) final BW, ADG, and G:F. A linear decrease ($P = 0.03$) was observed in DMI as DDG inclusion in the ration increased. Other trials have reported improvements in lamb ADG and DMI (Schauer et al., 2008; Crane et al., 2015) as concentration of DDG in the diet increased, most likely due to the increased nutrient concentration of the diet, specifically crude fat and CP. However, Crane et al. (2014) observed that when feeding corn-based feedlot diets with or without LAS, final BW and ADG were increased with no differences in G:F. The difference in treatment responses is likely due to the increased fiber content in the diets of the current trial with the inclusion of DDG. Associatively, G:F increased linearly ($P = 0.03$) as DDG in ration increased, showing a similar effect observed by Crane et al. (2015) in which the decreased DMI, but increased ADG drove the improved G:F; however, in the current trial, we observe no improvement in ADG other than by the inclusion of LAS.

There was no interaction ($P \geq 0.18$) of DDG and LAS inclusion for HCW, back fat, LEA, body wall thickness, flank streaking, lean maturity, yield grade, or % BCTRC; however conformation score was affected ($P = 0.005$) by the interaction of DDG and LAS. The majority of carcass traits were not affected by treatment (LAS or DDG inclusion level; $P \geq 0.07$). The inclusion of LAS increased ($P = 0.03$) HCW by 4%, similar to data reported by Crane et al. (2014) and was driven by the increase in ADG and final BW.

Digestibility Traits

Most observations for the N and S balance study were not affected ($P \geq 0.09$) by the inclusion of DDG, LAS, or the interaction of the two. A DDG x LAS interaction ($P = 0.04$) was observed for serum urea-N concentration, in which 0DDG-L and 15DDG-L were similar. However, the similar 0DDG-NL and 15DDG-L differed from the also similar 15DDG-NL, 30DDG-L, and 30DDG-NL treatments. Daily urinary sulfur excretion increased linearly ($P < 0.001$) with increasing inclusion rates of DDG in the diet. This is in agreement with Neville et al. (2010), who observed that lambs excrete substantial amounts of S when consuming DDG and that water intake and urinary output increase with increasing S intake. This could imply that although the NRC (2007) reports that feedlot type diets should not contain S in excess of 0.3% of the ration, it is possible that lambs merely excrete excess S, theoretically preventing a toxicity. Ruminal H_2S gas concentrations increased linearly ($P < 0.0001$) as concentration of DDG in the diet increased. Neville et al. (2010) also observed a linear increase in H_2S concentrations with increased inclusion rates of DDG in the diet; however, in the current trial no differences were observed for LAS fed treatments ($P = 0.82$).

Inclusion of LAS increased ($P = 0.02$) ruminal pH, retaining a slightly more basic rumen environment as well as exhibiting a tendency for a linear decrease ($P = 0.06$) with increased inclusion of DDG. However, Neville et al. (2010) observed no differences in pH in lambs fed similar diets to ours. Acetate exhibited a DDG x day interaction ($P = 0.03$; data not shown), with 15DDG fed lambs on d 23 having the highest concentration of acetate, similar to 0DDG on d 15, with it being a similar to all other concentrations. Acetate was expected to have a decrease due to LAS with propionate being increased as observed by Smith et al. (2010), however in the current trial, no LAS effects were observed on acetate or propionate ($P \geq 0.30$). Total VFA concentration in the rumen fluid decreased linearly ($P = 0.002$; Table 2) with increasing levels of DDG in the ration, possibly due to the increase in H_2S gas production leading to decreased microbial function. Kung et al. (2000) observed that excess dietary sulfur increased ruminal sulfide production; however it had no effect on VFA production. The results confirm our hypothesis that LAS increased overall growth and increasing DDG increased ruminal H_2S concentration, however, DDG inclusion did not increase growth. Additionally, we reject the

hypothesis that the combined effects of LAS and DDG would have no effect on rumen pH and VFA concentrations.

IMPLICATIONS

Dried distiller's grains with solubles decreased dry matter intake and improved feed conversion. The inclusion of lasalocid increased hot carcass weights by 4%, increasing lamb value for producers. Observations indicate when feeding increased levels of dried distiller's grains with solubles lambs may excrete excess sulfur in urine. Overall, dried distiller's grains with solubles appears to be a reliable lamb feedlot ration ingredient. Moreover, the combination of LAS and dried distiller's grains in the ration resulted in improved lamb feedlot performance.

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Table 1. Composition of diets fed to feedlot and digestibility trial lambs (DM basis)

	Dietary treatment[1]					
Item	ODDG-NL	0DDG-L	15DDG-NL	15DDG-L	30DDG-NL	30DDG-L
Ingredient, %						
DDG	-	-	14.8	14.8	29.7	29.8
Corn	79.9	79.9	65.2	65.2	50.2	50.3
MLM[2]	20.1	20.1	19.9	20.1	20.1	19.9
Nutritional composition						
Ash, %	5.8	6.0	6.3	6.9	7.8	7.2
TDN[3]	81.0	80.8	81.3	80.8	79.8	80.5
CP, %	16.7	17.3	16.5	16.6	16.7	16.9
ADF, %	4.0	3.9	5.8	5.8	9.8	9.3
Crude Fat, %	2.5	2.6	3.9	4.0	5.0	5.1
S, %	0.2	0.2	0.3	0.3	0.4	0.4
Ca, %	1.1	1.0	1.3	1.2	1.2	1.0
P, %	0.4	0.4	0.5	0.5	0.5	0.6

[1]Diets (DM basis) were balanced to be isonitrogenous and equal or greater than CP and NE requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007). DDG = dried distiller's grains with solubles; LAS = lasalocid. Treatments were: 0% DDG without LAS (0DDG-NL), 0% DDG with LAS (0DDG-L), 15% DDG without LAS (15DDG-NL), 15% DDG with LAS (15DDG-L), 30% DDG without LAS (30DDG-NL), and 30% DDG with LAS (30DDG-L).

[2]MLM = commercial market lamb meal contained 0.22 g/kg chlortetracycline, 38.0% CP, 3.75-4.75% Ca, 0.6% P, 3.0-4.0% salt, 1.2 mg/kg Se, 52,863 IU/kg vitamin A, 5,286 IU/kg vitamin D, and 209 IU/kg vitamin E, and LAS included into respective treatments at 20 g/ton.

[3]Calculated.

Table 2. Main effect means of increasing concentration of dried distiller's grains with solubles (DDG) with or without lasalocid (LAS, 20 g/ton) on lamb performance and digestibility

	Dietary treatment[1]							*P*-value[3]			
Item	ODDG-NL	0DDG-L	15DDG-NL	15DDG-L	30DDG-NL	30DDG-L	SEM[2]	DDG Linear	DDG Quadratic	LAS	DDG*LAS
Feedlot Performance											
Initial BW, kg	40.7	40.3	40.4	40.8	40.5	41.1	1.0	0.85	0.98	0.84	0.86
Final BW, kg	63.2	63.3	62.2	65.4	61.9	66.3	1.4	0.69	0.62	0.02	0.28
ADG, kg	0.28	0.29	0.27	0.30	0.27	0.31	0.009	0.57	0.82	<0.001	0.24
DMI, kg	1.8	1.8	1.9	1.6	1.6	1.5	0.1	0.03	0.37	0.15	0.52
G:F	0.07	0.08	0.07	0.09	0.08	0.10	0.005	0.03	0.67	0.003	0.28
Carcass Characteristics[4]											
HCW, kg	30.9	31.0	31.1	32.2	29.8	32.3	0.7	0.76	0.41	0.03	0.23
Back fat, cm	0.70	0.67	0.78	0.71	0.71	0.74	0.05	0.48	0.34	0.60	0.60
Loin Eye Area, cm^2	6.9	6.9	6.7	6.7	6.7	6.9	0.12	0.33	0.14	0.21	0.79
BWT, cm	2.5	2.3	2.5	2.5	2.5	2.5	0.09	0.16	0.25	0.58	0.37
Conformation	$959.7^{a,b}$	$975.7^{a,c}$	981.3^{c}	956.6^{b}	962.9^{a}	$971.8^{a,c}$	6.6	0.92	0.71	0.99	0.005
Flank Streaking	473.6	475.0	456.1	443.1	454.6	445.6	12.9	0.08	0.16	0.51	0.85
Lean Maturity	61.1	61.9	58.6	59.2	62.2	64.1	1.9	0.37	0.03	0.47	0.94
Yield Grade	3.2	3.1	3.5	3.2	3.2	3.3	0.2	0.48	0.34	0.60	0.60
BCTRC, %	43.3	43.5	43.2	43.0	43.5	43.1	0.2	0.39	0.20	0.21	0.28
Digestibility Traits, g/kg BW											
Daily DMI	26.3	28.4	27.0	30.7	26.2	29.1	4.9	0.95	0.74	0.48	0.99
Daily N intake	0.67	0.75	0.73	0.82	0.72	0.81	0.13	0.63	0.74	0.44	0.99
Daily N excretion											
Fecal	0.13	0.15	0.15	0.17	0.15	0.18	0.04	0.57	0.74	0.52	0.99
Urinary	0.03	0.04	0.03	0.03	0.04	0.04	0.004	1.00	0.70	0.20	0.93
Daily N balance	0.57	0.63	0.60	0.69	0.60	0.67	0.1	0.70	0.75	0.39	0.99
Daily S intake	0.17	0.18	0.17	0.19	0.14	0.18	0.03	0.66	0.58	0.40	0.84
Daily S excretion											
Fecal	0.011	0.014	0.015	0.015	0.015	0.018	0.004	0.23	0.85	0.94	0.94
Urinary	0.003	0.003	0.004	0.005	0.006	0.007	0.0008	<0.001	0.93	0.73	0.73
Daily S Balance	0.17	0.16	0.16	0.18	0.14	0.17	0.03	0.66	0.59	0.77	0.77
Serum-N, mg/dL[5]	17.1^{b}	19.2^{a}	14.5^{c}	$17.6^{a,b}$	13.6^{c}	13.9^{c}	0.6	<0.001	0.92	0.04	0.04
Serum-S, mg/dL[5]	83.2	88.2	88.4	83.78	85.3	87.2	2.1	0.86	0.96	0.09	0.09
H_2S, ppm[6]	0.005	0.012	0.082	0.056	0.190	0.195	0.02	<0.001	0.12	0.74	0.74
Rumen Fluid pH	4.96	5.08	4.9	5.08	4.85	4.92	0.06	0.06	0.52	0.69	0.69
Total VFA	3506.7	3403.0	2796.5	2966.3	1798.4	2745.0	341.9	0.002	0.95	0.29	0.23

[1]Diets (DM basis) were balanced to be isonitrogenous and ≥ CP and NE requirements of a 40 kg lamb gaining 300 g/d (NRC, 2007). Treatments were: 0% DDG without LAS (0DDG-NL), 0% DDG with LAS (0DDG-L), 15% DDG without LAS (15DDG-NL), 15% DDG with LAS (15DDG-L), 30% DDG without LAS (30DDG-NL), and 30% DDG with LAS (30DDG-L).

[2]n = 6; SEM = standard error of the means for main effects.

[3]*P*- value for linear and quadratic effects of increasing DDG inclusion in the diet and main effects of DDG, LAS, and DDG*LAS.

[4]BWT= Body wall thickness; Conformation: 100 = cull to 1500 = high prime. Flank Streaking: 100 to 199 = practically devoid; 200 to 299 = traces; 300 to 399 = slight; 400 to 499 = small; 500 to 599 = modest. Lean maturity: 0 = very fine to 100 = fine. Yield grade = 0.4 + (10 × fat depth). BCTRC = boneless closely trimmed retail cuts, % = [49.936- (0.0848 × 2.205 × HCW, kg) – (4.376 × 0.3937 × back fat, cm) – (3.53 × 0.3937 × body wall thickness, cm) + (2.456 × 0.155 × loin eye area, cm^2)].

[5]*P*-values for serum urea-N: day (P = 0.08) and treatment × day (P <0.001); serum-S was only analyzed on d 18 of the digestibility trial.

[6]H_2S = hydrogen sulfide gas. *P*-values for H_2S gas: day (P = 0.30) and treatment × day (P <0.001).

[a,b,c]Means within a row with different superscripts differ ($P \leq 0.05$).

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Impacts of stocking density on growth and puberty attainment of replacement beef heifers

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ABSTRACT: Sixty Angus x Hereford heifers were ranked by age and BW (210 ± 2 d and 220 ± 2 kg, respectively) on d 0, and assigned to: A) 1 of 3 drylot pens (10 × 14 m pens; 10 heifers/pen) resulting in a stocking density of 14 m^2/heifer (**HIDENS**), or B) 1 of 3 pastures (1-ha pastures; 10 heifers/pasture), resulting in a stocking density of 1,000 m^2/heifer (**LOWDENS**). These treatments were designed to represent typical commercial US cow-calf system stocking densities. Prior to the beginning of the experiment, pastures were harvested for hay leaving no forage available for grazing for LOWDENS heifers. All heifers received the same diet consisting of (as-fed basis) 5 kg alfalfa hay and 3.5 kg of corn per heifer/d. On d 0, heifers were fitted with a pedometer (HJ-321; Omron Healthcare, Inc., Bannockburn, IL). Each week for the duration of the experiment (d 0 to d 161), pedometer results were recorded, heifer BW was measured, and blood samples were collected. Heifer shrunk BW (after 16 h of water and feed deprivation) was also collected on d -5 and 162 of the experiment. Puberty onset was determined according to plasma progesterone concentration. Heifers were considered pubertal when plasma progesterone concentration was greater than 1.0 ng/mL for 2 consecutive weeks. A treatment x day interaction was detected ($P < 0.01$) for BW, considering HIDENS heifers were on average 10 ± 4 kg heavier than LOWDENS heifers beginning on day 28. This difference in body weight can be attributed to increased physical activity of LOWDENS heifers, as they exhibited more ($P < 0.01$) steps compared to HIDENS heifers. However, ADG using shrunk BW values did not differ ($P = 0.49$) among treatments. Treatment x day interactions were detected for plasma cortisol ($P < 0.01$) and IGF-1 ($P < 0.01$), given that concentration of these hormones were greater for LOWDENS compared to HIDENS heifers on d 84 ($P < 0.01$) and d 140 ($P \leq 0.04$). A treatment x day interaction was also detected ($P < 0.01$) for puberty attainment, considering a greater proportion of LOWDENS heifers reached puberty compared with HIDENS cohorts during the experiment (54.6 vs. 3.4% of heifers pubertal by d 161, respectively; $P < 0.01$, SEM = 5.3). In conclusion, heifers reared in a low-stocking density exhibited hastened puberty attainment, despite the observed decrease in heifer BW attributed to increased physical activity, compared with heifers reared in high stocking density.

Keywords: Beef heifers, growth, puberty, stocking density

INTRODUCTION

Public scrutiny on beef production systems is growing rapidly, and cattle welfare is one of the main targets for attention (Grandin, 2014). Cattle producers are challenged to improve their productive efficiency while assuring proper animal care. Hence, management that increase beef cattle productivity and promote animal welfare are warranted to: 1) enhance profitability in beef cattle systems, 2) address the current and projected increases in beef demand, and 3) satisfy industry and public requirements for proper animal care.

Stocking density is one example of management that may impact welfare and productive efficiency in beef operations. In spring-calving cow-calf herds, replacement heifers are weaned in the fall and exposed to their first breeding season the following spring/summer. Therefore, it is common for these heifers to be reared in drylot systems during the fall/winter/early spring to facilitate management and supply of feed and water. However, rearing cattle in confined areas is known to stimulate stress reactions (Grandin, 2014), while acute and chronic stress directly impairs reproductive function in beef cattle (Dobson and Smith, 2000). Petersen et al. (2014) reported that heifers developed on drylot (11 m^2/heifer) gained more weight but had increased heart rate and rested less compared with contemporary heifers reared on native range (7,400 m^2/heifer), corroborating that rearing heifers in confined spaces is indeed detrimental to their welfare. Mulliniks et al. (2013) reported that heifers reared in drylots had greater ADG, but reduced pregnancy rates compared with cohorts reared on range pastures.

Therefore, we hypothesized that elevated stocking density impairs reproductive development in beef heifers. To test our hypothesis, the objective of this experiment was to compare growth, physical activity, stress-related responses, and puberty attainment in heifers reared on high- or low-stocking densities from weaning until 12 mo of age.

MATERIALS AND METHODS

This experiment was conducted at the Oregon State University - Eastern Agricultural Research Center (Burns, OR) from September 2015 until March 2016. All animals were cared for in accordance with accepatable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee (#4757).

Animals. On day 0, 60 Angus x Hereford weaned heifers were ranked by age and BW (210 ± 2 d and 220 ±

2 kg, respectively) and allocated to: A) 1 of 3 drylot pens (10 x 14 m pens; 10 heifers/pen), resulting in a stocking density of 14 m^2/heifer (**HIDENS**), or B) 1 of 3 pastures (1-ha pastures; 10 heifers/pasture), resulting in a stocking density of 1,000 m^2/heifer (**LOWDENS**). Treatments were designed to represent stocking densities typical of commercial US cow-calf systems. Prior to the beginning of this experiment, pastures were harvested for hay; therefore no pasture was available for grazing for LOWDENS heifers. All heifers received the same diet, consisting of (as-fed basis) 5 kg of alfalfa hay and 3.5 kg of corn per heifer daily. Diets were completely consumed by heifers within 24 h after being offered. On d 0, heifers were fitted with a pedometer (HJ-321; Omron Healthcare, Inc., Bannockburn, IL) placed inside a polyester patch (Heat Watch II; Cow Chips, LLC, Manalapan, NJ) fixed behind their right shoulder to assess physical activity.

Sampling. Shrunk BW was recorded after 16 h of feed and water withdrawal on d -5 and d 162 for ADG calculations. Each week during the experiment (day 0 to 161), pedometer results were recorded, heifer full BW was measured, and blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson Franklin Lakes, NJ) with 158 USP units of freeze-dried sodium heparin for plasma collection. All blood samples were placed immediately on ice, centrifuged (2,500 x g for 30 min; 4°C) for plasma harvest, and stored at -80°C.

Plasma progesterone, cortisol, and IGF-1 concentrations were analyzed using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Heifers were considered pubertal when plasma progesterone concentration was greater than 1.0 ng/mL for 2 consecutive weeks (Perry et al., 1991), and puberty attainment was determined at the second week with elevated progesterone.

Statistical Analysis. All data were analyzed using pen or pasture (3 replications/treatment) as experimental unit, with the MIXED or GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, NC, USA) for quantitative and binary data, respectively, and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. All data were analyzed using replication(treatment) and heifer(replication) as random effects. The model statement used for ADG, initial and final BW contained the effects of treatment. The model statement for weekly BW, puberty attainment, steps, and plasma hormones contained the effects of treatment, day, and the treatment × day interaction. The specified term for the repeated statement was day, with heifer(replication) as subject. Results are reported as least square means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to effect of treatment if no interactions are significant, or according to the highest order interaction detected.

RESULTS AND DISCUSSION

No treatment effect was detected ($P > 0.40$) for the analysis of initial BW, final BW, or ADG (Table 1). However, a treatment x day interaction was detected ($P < 0.01$) for weekly BW, since HIDENS heifers were on average 10 ± 4 kg heavier beginning on day 28 compared to LOWDENS heifers (Figure 1). This can be attributed to greater physical activity, as measured by steps/week, of LOWDENS heifers given these had more ($P < 0.01$) steps compared with HIDENS heifers (Table 1).

Table 1. Growth parameters and activity of heifers reared in low stocking density (1,000 m^2/heifer; **LOWDENS**) or high stocking density (14 m^2/heifer; **HIDENS**)

Item	LOWDENS	HIDENS	SEM	*P* =
Growth parameters				
Initial BW, kg	211	212	3	0.78
Final BW, kg	343	347	5	0.59
ADG, kg/d	0.79	0.82	0.02	0.49
Activity				
Steps/week	18,580[a]	2,775[b]	595	< 0.01

Treatment x day interactions were ($P < 0.01$) detected for plasma cortisol and IGF-I (Figure 2). Plasma concentrations of both hormones were greater for LOWDENS compared to HIDENS heifers on d 84 ($P < 0.01$) and d 140 ($P \leq 0.04$). Previous research reported that both IGF-I and cortisol are increased in plasma in response to physical activity (Schwarz et al., 1996; Raastad et al., 2000; Hill et al., 2008). Hence, treatment differences in plasma cortisol and IGF-I concentrations on d 84 and 140 can be attributed, at least partially, to the activity of gathering and bringing the LOWDENS heifers from pasture to the working facility, whereas HIDENS heifers were grouped in drylot pens located near the working facility.

A treatment x day interaction was detected ($P < 0.01$) for puberty attainment, considering a greater proportion of LOWDENS heifers reached puberty during the experiment compared with HIDENS heifers (Figure 3). The mechanism by which stocking density affects puberty attainment is unclear, however physical activity should be considered. Lamb et al. (1979) reported dairy heifers exposed to forced exercise were more efficient reproductively, even though no change in BW was observed. Physical activity causes release of endogenous opioids (Grossman et al., 1984), and there is evidence that these opioids play a role in regulation of gonadotrophin secretion (Mahmoud et al., 1989; Evans et al., 1992). Therefore, the additional physical activity exhibited by LOWDENS heifers may have contributed to their increased rate of puberty attainment.

Traditionally, BW has been thought to play a pivotal role in heifer reproductive maturation (Patterson et al., 1992). Endocrine activity involved in puberty appears to be suppressed until the heifer is of a sufficient size to be successful reproductively (Rawlings et al., 2003). Given the HIDENS heifers in this experiment were on average 10 ± 4 kg heavier beginning on day 28 compared to LOWDENS heifers, and yet failed to reach puberty at the same rate as LOWDENS heifers (3.4 vs. 54.6% of heifers pubertal by d 161, respectively), BW and nutritional status do not appear

to be the only regulators of hormonal stimulation required for puberty onset.

IMPLICATIONS

Results from this experiment indicate that stocking density impacts puberty attainment in beef heifers. Heifers reared in a low-stocking exhibited hastened puberty attainment, despite the often decrease in BW recorded weekly, which can be attributed to their increased physical activity compared with heifers reared in a higher stocking density. Therefore, rearing heifers in a low-stocking density may be a strategy to increase rate of puberty attainment, and hence efficiency of cow-calf operations.

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Figure 1. Body weight change in heifers reared in low stocking density (1,000 m^2/heifer; **LOWDENS**) or high stocking density (14 m^2/heifer; **HIDENS**). A treatment × day interaction was detected ($P < 0.01$). Within days, * $P \leq 0.05$, ** $P \leq 0.01$.

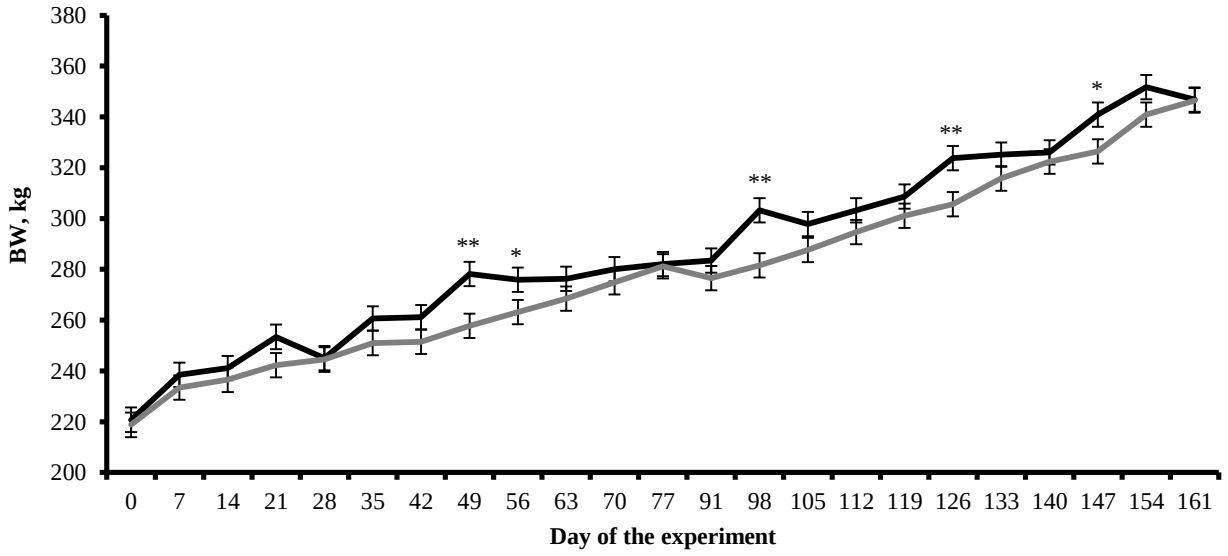


Figure 2. Plasma concentrations of cortisol (Panel A) and IGF-I (Panel B) from heifers reared in low stocking density (1,000 m^2/heifer; **LOWDENS**) or high stocking density (14 m^2/heifer; **HIDENS**). A treatment × day interaction was detected ($P < 0.01$). Within days, * $P \leq 0.05$, ** $P \leq 0.01$.

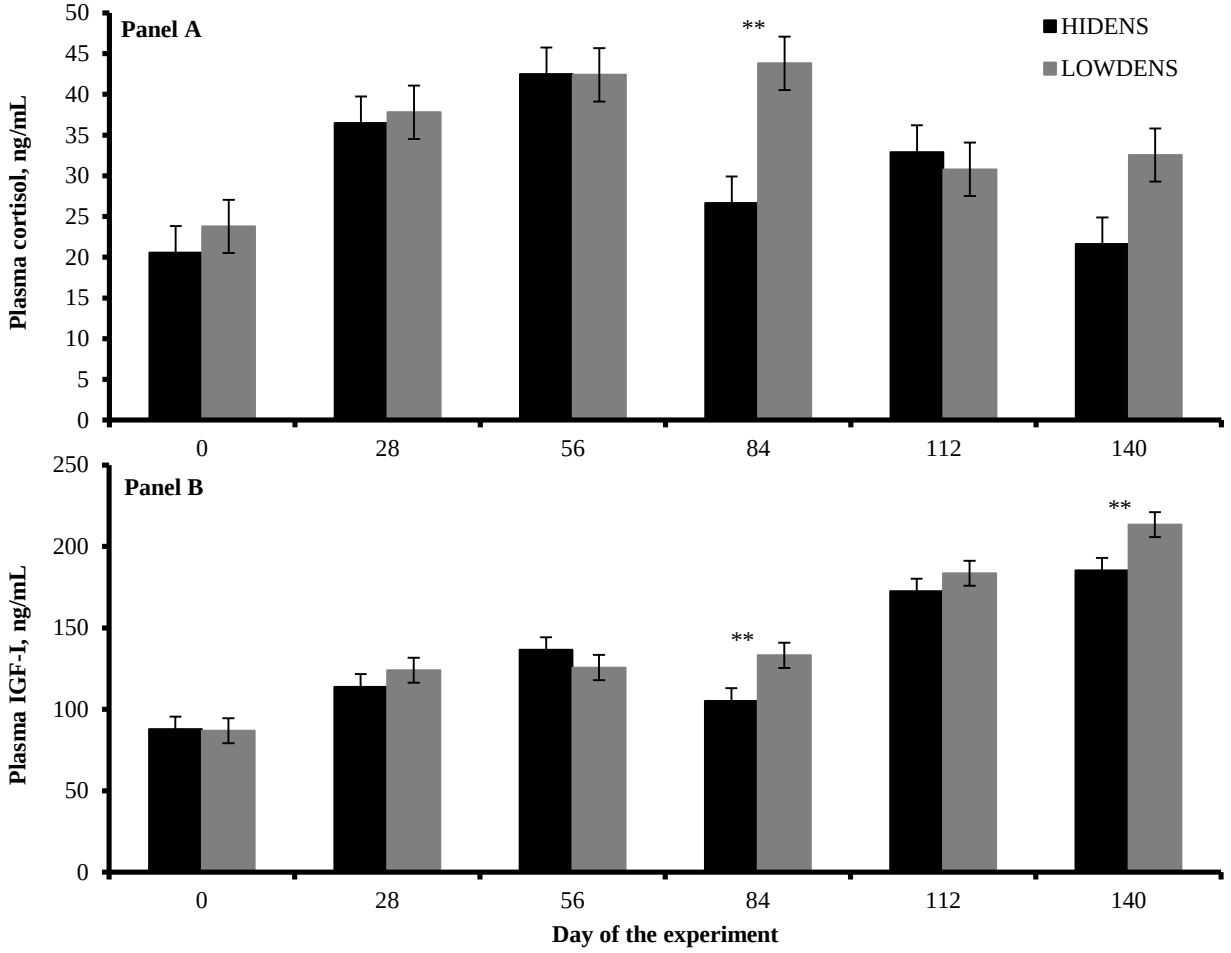
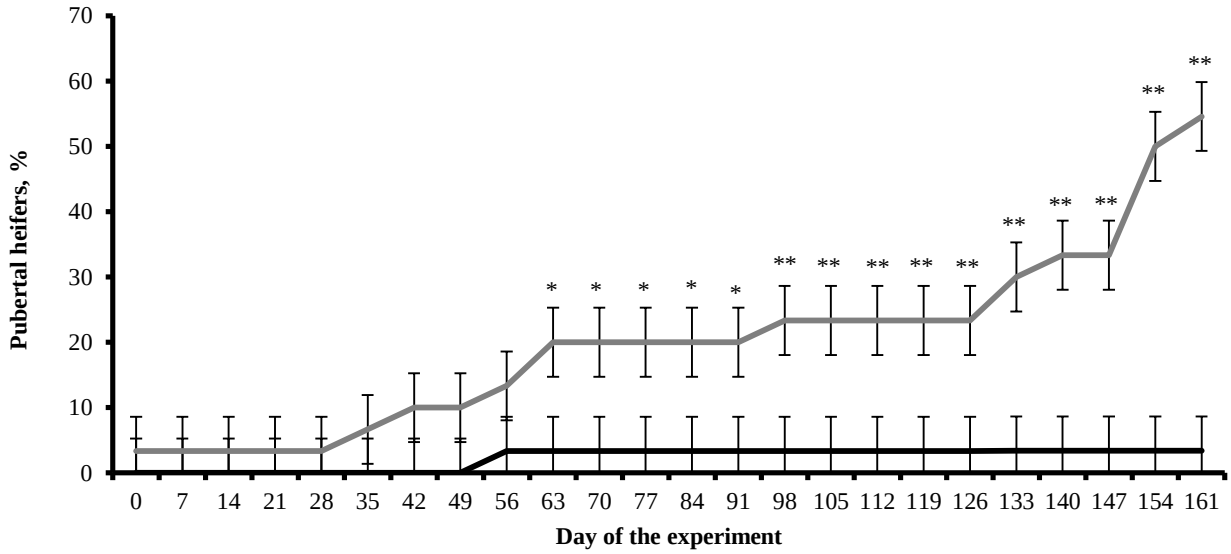


Figure 3. Puberty attainment in heifers reared in low stocking density (1,000 m^2/heifer; **LOWDENS**) or high stocking density (14 m^2/heifer; **HIDENS**). A treatment × day interaction was detected ($P < 0.01$). Within days, * $P \leq 0.05$, ** $P \leq 0.01$.



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Physiologic, health, and production responses of dairy cows supplemented with an immunomodulatory feed ingredient during the transition period

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Abstract text: This study compared physiological, health, and productive parameters in dairy cows supplemented or not with Omnigen-AF (**OMN**; Phibro Animal Health, Teaneck, NJ) during the transition period. Thirty-eight non-lactating, multiparous, pregnant Holstein × Gir cows were ranked by BW and BCS, and assigned to receive (n = 19) or not (**CON**; n = 19) OMN at 56 g/cow daily (as-fed basis) beginning 35 d prior to expected date of calving. Before calving, cows were maintained in single drylot pen with ad libitum access to corn silage, and received (as-fed basis) 3 kg/cow daily of a concentrate. After calving, cows were moved to an adjacent drylot pen, milked twice daily, offered (as-fed basis) 35 kg/cow daily of corn silage, and individually received a concentrate formulated to meet their nutritional requirements after both milkings. Cows received OMN individually as top-dressing into the morning concentrate feeding. Before calving, cow BW and BCS were recorded weekly and blood samples collected every 5 d beginning on d -35 relative to expected calving date. After calving and until 46 days in milk (**DIM**), BW and BCS were recorded weekly, individual milk production was recorded and milk samples were collected daily for total solids and somatic cell count analyses. Blood was sampled daily from 0 to 7 DIM, every other day from 9 to 21 DIM, and every 5 d from 26 to 46 DIM. On 30 and 46 DIM, cows were evaluated for endometritis via cytobrush technique, based on % of polymorphonuclear (**PMN**) cells in 100 total cell count (PMN + endometrial cells). On 48.7 ± 1.6 DIM, 9 cows/treatment received a lipopolysaccharide (**LPS**) injection (0.25 μg/kg of BW), and blood was sampled hourly from -2 to 8 h, at 12-h intervals from 12 to 72 h, and at 24-h intervals form 96 to 120 h relative to LPS administration. No treatment differences were detected on BW, BCS, serum concentrations of cortisol, NEFA, insulin, glucose, haptoglobin, cortisol, and IGF-I ($P \geq 0.15$). Cows receiving OMN had greater ($P \leq 0.04$) milk yield (30.3 vs. 27.1 kg/d; SEM = 0.9) and percentage of PMN cells in endometrial cell population (12.2 vs. 3.9%; SEM = 2.9) compared with CON cows. After LPS administration, cows receiving OMN had greater ($P \leq 0.04$) mean serum haptoglobin (212 vs. 94 μg/mL; SEM = 38), as well as greater serum concentration of tumor necrosis factor alpha at 1, 2, and 3 h relative to LPS injection compared with CON cows. In conclusion, OMN supplementation during the transition period enhanced innate immunity parameters and increased milk production in dairy cows.

Keywords: Inflammation, milk production, Omnigen-AF, transition cows.

INTRODUCTION

During the transition period, dairy cows experience physiological changes associated with parturition and onset of lactation that impair their immune function (Mallard et al., 1998). These include increased lipolysis, altered insulin-glucose and somatrotopic axes, as well as heightened inflammatory and acute-phase reactions (Grummer, 1995; Sheldon et al., 2001). Consequently, transition dairy cows are highly susceptible to metabolic and infectious diseases that directly impact their lactation productivity and well-being (Mallard et al., 1998). Hence, management strategies that modulate physiology and enhance immunocompetence of transition dairy cows are warranted to optimize profitability in dairy production systems (Overton and Waldron, 2004).

Omnigen-AF® (**OMN;** Phibro Animal Health, Teaneck, NJ) is a patented proprietary branded product shown to modulate innate and adaptive immune function in ruminants and other livestock species. More specifically, previous research suggested heightened innate immunity when OMN is supplemented (Wang et al., 2009; Ryman et al., 2013), although the impacts of this feed additive on metabolic and productive responses of transition dairy cows still warrants investigation. Based on this rationale, we hypothesized that OMN supplementation to dairy cows will optimize metabolic and innate immune responses during the transition period, resulting in enhanced milk production. Therefore, this experiment compared physiological, health, and productive parameters in dairy cows supplemented or not with OMN prior to calving and during early lactation.

MATERIALS AND METHODS

Animals and treatments. Animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the São Paulo State University - Animal Ethics Committee. Thirty-eight non-lactating, multiparous, pregnant Holstein × Gir cows (BW = 638 ± 12 kg, BCS = 3.33 ± 0.08) were ranked by parity, BW, and BCS and assigned to receive (n = 19) or not (**CON**; n = 19) 56 g/cow daily of OMN beginning 35 d prior to expected calving date. Cows were maintained in a single drylot pen throughout the experiment. Prior to calving, cows had ad libitum access to corn silage, water, and a commercial pre-partum mineral mix and individually received 3 kg/cow daily of a concentrate (Table 1) through self-locking head gates once daily (0800 h). After calving,

cows had ad libitum access to water and a commercial lactation mineral mix. Cows were milked twice daily (0600 and 1700 h), cows were group-fed (as-fed basis) 35 kg/cow daily of corn silage and individually received a concentrate (Table 1) through self-locking head gates immediately after both milkings. Concentrate offer was adjusted weekly using the Spartan Dairy Ration Evaluator/Balancer (version 3.0; Michigan State University, East Lansing, MI). The OMN was top-dressed daily into the morning concentrate feeding. Samples of the offered corn silage, pre-partum, and lactation concentrates were collected every 2 wks, and analyzed for nutrient content by a bromatology laboratory (3rlab, Belo Horizonte, Brazil). Calculations of ME, NE_L, and NE_M used the equation proposed by the NRC (2001). Concentration of DM was 38.4% in corn silage, 90.1% in pre-partum concentrate, and 89.0% in lactation concentrate. Nutritional profile (DM basis) of corn silage was 53% NDF, 33% NFC, 2.24 Mcal/kg of ME, 1.39 Mcal/kg of NE_L, 1.39 Mcal/kg of NE_M, and 8.1% CP.

Cow BW and BCS were recorded once weekly throughout the experiment. Prior to calving blood samples were collected every 5 d beginning on d -25 relative to expected calving date immediately prior to concentrate feeding (0800 h). After calving, individual milk production was recorded daily until d 46 of lactation. Milk samples were collected daily from each cow and analyzed for somatic cell count (**SCC**) and for total solids content by a commercial laboratory (Clínica do Leite; Universidade de São Paulo, Piracicaba, Brazil). Blood samples were collected daily from d 0 to 7 of lactation, every other day from d 9 to 21 of lactation, and every 5 d from d 26 to 46 of lactation, immediately prior to morning concentrate feeding (0600 h). On d 30 and 46, cows were evaluated for endometritis via cytobrush technique (VWR CanLab, Mississauga, Ontario, Canada) according to the procedures reported by Dubuc et al. (2010). Slides for cytological examination were immediately stained with a panoptic stain (Insta-Prov; Newprov, Pinhais, PR, Brazil), and stored at room temperature until further analysis. Each slide was examined as described by Dubuc et al. (2010). Results are reported as percentage of polymorphonuclear (**PMN**) cells within 100 total cell count (PMN + endometrial cells).

At the end of the experiment, 9 cows were randomly selected from each treatment group and assigned to a lipopolysaccharide (**LPS**) challenge. Selected cows received an intravenous bolus dose of bacterial LPS (0.25 μg/kg of BW, *Escherichia coli* 0111:B4, Sigma-Aldrich Brasil Ltda., São Paulo, Brazil) on d 48.7 ± 1.6 of lactation. Blood samples were collected hourly from -2 to 8 h, at 12-h intervals from 12 to 72 h, and at 24-h intervals form 96 to 120 h relative to LPS administration.

All blood samples were obtained from either the coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ), placed immediately on ice, centrifuged at 3000 × g at 4 °C for 30 min for serum collection, and stored at -20°C on the same day of collection. Pre- and post-calving samples were analyzed for serum concentrations of glucose, NEFA, haptoglobin, IGF-I, cortisol and insulin. Samples collected during the LPS challenge were analyzed for haptoglobin and tumor necrosis factor alpha (**TNF*α***).

Statistical analysis. Quantitative and binary data were analyzed, respectively, with the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc.; version 9.3), using cow as the experimental unit and cow(treatment) as random variable. The model statement used for analysis of BW and BCS change, as well as initial, post-calving, and final BCS and BW during the experiment contained the effects of treatment. The model statement used for analysis of weekly BW and BCS contained the effects of treatment, week, and the resultant interaction. The model statement used for analysis of daily milk production, daily concentrate intake, cytobrush results, and serum variables contained the effects of treatment, day (or hour for the LPS challenge), and the resultant interaction. Daily milk production was also analyzed according to percentage of PMN cells during the experiment, and this model contained the effects of treatment, PMN ranking (≥ 6% or < 6% PMN cells), day, and the resultant interactions. The specified term for the repeated statements was week for BCS and BW, hour for LPS variables, and day for the remaining analysis, with cow(treatment) as subject. The covariance structure utilized for all repeated statements was compound symmetry, which provided the best fit for these analyses according to the Akaike information criterion. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results were separated using LSD, and are reported as least square means according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

RESULTS AND DISCUSSION

No treatment × day or week interactions were detected ($P \geq 0.36$) for BW, BCS, and serum variables during pre- and post-calving. No treatment effects were detected on BW ($P \geq 0.15$) and BCS ($P \geq 0.55$) parameters upon calving, or throughout the experimental period (data not shown). No treatment effects were also detected ($P \geq 0.17$) for serum concentrations of NEFA, insulin, glucose, and IGF-I (Table 2). The published literature on OMN supplementation to dairy cows is limited, and no other research has evaluated its impacts on body and physiological parameters during the transition period. Nevertheless, these results are indicative that OMN supplementation did not modulate the nutritional and metabolic challenges caused by calving and beginning of lactation evaluated herein (Jorritsma et al., 2003).

No treatment effects were detected ($P \geq 0.62$) for serum cortisol and haptoglobin concentrations (Table 2). These outcomes suggest that both treatments experienced a similar corticosteroid and acute-phase protein reaction elicited by stress, trauma, injuries, and inflammation associated with parturition and onset of lactation (Cray et al., 2009). Others have reported that OMN supplementation modulated innate immune reactions in dairy (Wang et al., 2009), whereas cortisol and haptoglobin are major components of the innate immune system (Carroll and Forsberg, 2007). However, these research efforts focused on leukocyte-specific variables, as well as oxidative stress and phagocytic activity upon a pathogenic challenge (Ryman et al., 2013). Although the present experiment did

not evaluate leukocyte mRNA and activity variables, the lack of treatment effects on cortisol and haptoglobin reported herein suggest that the potential cell-based immune benefits of OMN supplementation did not modulate the neuroendocrine stress and acute-phase protein reactions triggered by calving and beginning of lactation.

Cows supplemented with OMN had greater (P = 0.02) milk yield compared with CON cows (Table 3). No differences were detected ($P \geq 0.12$) for milk total solids concentration and SCC, whereas OMN-supplemented cows tended ($P = 0.07$) to have greater 12% total solids-corrected milk compared with CON cohorts (Table 3). The lack of a treatment × day interaction ($P = 0.99$) for milk yield indicates that OMN-supplemented cows produced more milk as soon as lactation started and such difference persisted until 46 DIM, suggesting that the productive benefits of OMN supplementation are associated with its pre- and postpartum supplementation. It is important to note that milk production is directly impacted by feed intake (NRC, 2001), which was not fully assessed in the present experiment. Concentrate was provided equally to OMN-supplemented and CON cows prior to calving, and postpartum concentrate DMI was similar ($P = 0.37$) among treatments (7.6 vs. 8.0 kg/d on as-fed basis for CON and OMN-supplemented cows, respectively; SEM = 0.35). Moreover, serum concentrations of glucose, NEFA, insulin, and IGF-I are typical markers of nutrient intake in dairy cattle (Butler, 2003), and did not differ ($P \geq 0.17$) between OMN-supplemented and CON cows (Table 2). Hence, results from this experiment indicate that the effects of OMN supplementation on milk yield of transition dairy cows appear to be independent of the metabolic parameters evaluated herein.

Cows receiving OMN had greater ($P = 0.04$) percentage of PMN cells in endometrium cell population compared with CON cows (12.2 vs. 3.9%, respectively; SEM = 2.9). Others have utilized the cytobrush technique and subsequent PMN cell count as indicators of endometritis in dairy cattle (Dubuc et al., 2010), and positively associated PMN cell count with plasma haptoglobin concentrations and decreased milk yield (Burke et al. 2010). In the present experiment, however, OMN-supplemented cows had greater milk production and similar serum haptoglobin concentrations compared with CON cows, despite treatment difference in PMN cells. One of the cell-based immune benefits attributed to OMN is to increase L-selectin mRNA expression in leukocytes (Ryman et al., 2013); an adhesion molecule that allows leukocytes to migrate from capillaries into infected tissues to phagocytize and kill bacteria (Abbas and Lichtman, 2007). Therefore, one can speculate that the greater percentage of PMN cells detected in OMN-supplemented cows does not represent increased incidence of endometritis, but is indicative of greater capability of leukocyte migration into their endometrium compared with CON cohorts. Accordingly, Yasui et al. (2014) suggested that early infiltration of PMNL into the uterus post-calving may be indicative of robust immune function, and an important factor in reducing subsequent incidence of endometritis in dairy cows.

To support this latter rationale, OMN-supplemented and CON cows were ranked according to average (d 30 and 46) percentage of PMN cells during the experiment and classified as ≥ 6% or < 6% PMN cells, which is a criterion previously used for cytological endometritis (Dubuc et al., 2010). A treatment × PMN group × day interaction was detected ($P = 0.04$; Figure 1) for milk yield. Beginning on d 14 of lactation, milk yield in CON cows with ≥ 6% PMN cells was often reduced ($P < 0.05$) vs. CON cows with < 6% PMN cells, as well as vs. OMN-supplemented cows with ≥ 6% or < 6% PMN cells (average milk yield from 14 to 46 DIM = 25.7, 29.9, 31.4, and 32.1 kg/d, respectively; SEM = 1.5). In addition, milk yield between CON cows with ≥ 6% PMN cells was similar ($P \geq 0.21$) compared to milk yield of both OMN-supplemented groups. Given that uterine infection impairs milk production (Lewis, 1997), these results corroborate that treatment differences on percentage of PMN cells was indicative of enhanced immune response in OMN-supplemented cows. In addition, these results indicate that treatment effects detected for milk yield (Table 3) were largely influenced by productivity of CON cows with ≥ 6% PMN cells, suggesting that OMN supplementation increased milk yield by enhancing immunocompetence of post-partum dairy cows.

Upon a pathogenic stimulus such as LPS challenge, the innate immune system elicits several reactions with the intent of controlling or eliminating the infection (Abbas and Lichtman, 2007). These include synthesis of proinflammatory cytokines such as TNFα and hepatic synthesis of acute phase proteins including haptoglobin (Carroll and Forsberg, 2007). Accordingly, time effects ($P < 0.01$) were detected for serum TNFα, and serum haptoglobin during the LPS challenge period (Figure 2), indicating that LPS administration elicited the expected innate immune responses (Carroll and Forsberg, 2007). In addition, a treatment × hour interaction was detected ($P = 0.03$) for serum TNFα concentration, which was greater ($P \leq 0.04$) for OMN-supplemented cows compared with CON cows at 1, 2, and 3 h relative to LPS administration (Figure 2). A treatment effect was detected for serum haptoglobin concentration, which was greater ($P = 0.04$) in OMN-supplemented cows compared with CON cows during the entire LPS challenge period (212 vs. 94 μg/mL, respectively; SEM = 38). However, serum haptoglobin concentration was only greater ($P \leq 0.05$) in OMN-supplemented vs. CON cows from 36 to 72 h after LPS challenge (Figure 2), when serum haptoglobin is expected to peak upon LPS administration (Rodrigues et al., 2015), although the treatment × hour interaction was not significant ($P = 0.50$). Supporting our results, Burdick et al. (2012) also reported increased acute-phase reaction in beef cattle supplemented with OMN and receiving LPS administration. Therefore, OMN supplementation enhanced innate immune reactions elicited upon LPS administration with the intent of restoring homeostasis (Abbas and Lichtman, 2007). These results are in agreement with the immune benefits attributed to OMN by others (Ryman et al., 2013), including treatment effects detected herein for percentage of PMN cells in the endometrium.

IMPLICATIONS

Collectively, results from this experiment indicate that the OMN supplementation enhanced immunocompetence of transition dairy cows, resulting in increased performance. More specifically, supplementing OMN did not impact metabolic and acute-phase protein responses triggered by calving and beginning of lactation, but increased milk yield, percentage of PMN cells in the endometrium, as well as TNFα and haptoglobin response upon LPS administration. Hence, OMN supplementation during the transition period appears to be an effective approach to improve cow welfare and productivity in dairy production systems.

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Table 1. Composition and nutritional profile of concentrate offered to transition dairy cows prior and after calving

Item	Pre-partum	Lactation
Composition (% DM basis)		
Ground corn	45.5	56.8
Soybean meal	45.5	40.5
Pre-partum mineral mix[1]	9.0	0.0
Lactation mineral mix[2]	0.0	2.7
Nutrient profile (%DM basis)		
NDF	12.0	13.0
NFC	58.0	58.0
ME	2.76	2.99
NE_L	1.80	1.92
NE_M	1.91	2.04
CP	24.2	23.1

[1] Milk Ionic (M. Cassab Tecnologia Animal, São Paulo, Brazil).
[2] Milk MAC (M. Cassab Tecnologia Animal).

Table 2. Serum parameters of dairy cows supplemented with Omnigen-AF (**OMN**; n = 19) or not (**CON**; n = 19) prior to and for 46 d after calving.

Item	OMN	CON	SEM	*P* =
Cortisol, ng/mL	10.9	10.3	0.82	0.62
Glucose, mg/dL	55.6	56.4	0.97	0.56
Haptoglobin, µg/mL	256	226	44	0.64
IGF-I, ng/mL	56.9	68.3	5.8	0.17
Insulin, pmol/l	3.82	0.83	2.01	0.30
NEFA, µEq/L	0.421	0.369	0.036	0.31

[1] Blood samples were scheduled to be collected every 5 d beginning on d -25 relative to expected calving date (d -25, -20, -15, -10, and -5). According to actual calving dates, samples collected were rounded into the nearest pre-scheduled sampling date. After calving, blood samples were collected daily from d 0 to 7 of lactation, every other day from d 9 to 21 of lactation, and every 5 d from d 26 to 46 of lactation.

Figure 1. Milk yield of dairy cows supplemented with Omnigen-AF (**OMN**; n = 19) or not (**CON**; n = 19) prior to and for 46 d after calving, and classified according to percentage of polymorphonuclear (**PMN**; ≥ 6% or < 6%; criterion for cytological endometritis) within 100 total cell count (PMN + endometrial cells) from endometrial samples collected via cytobrush technique on d 30 and 46 of lactation (according to Dubuc et al., 2010). A treatment × PMN group × day interaction was detected ($P = 0.04$). Within days, CON + PMN ≥ 6% cows had (* = $P \leq 0.05$; † = $P \leq 0.10$) reduced milk yield compared with all other treatment combinations.

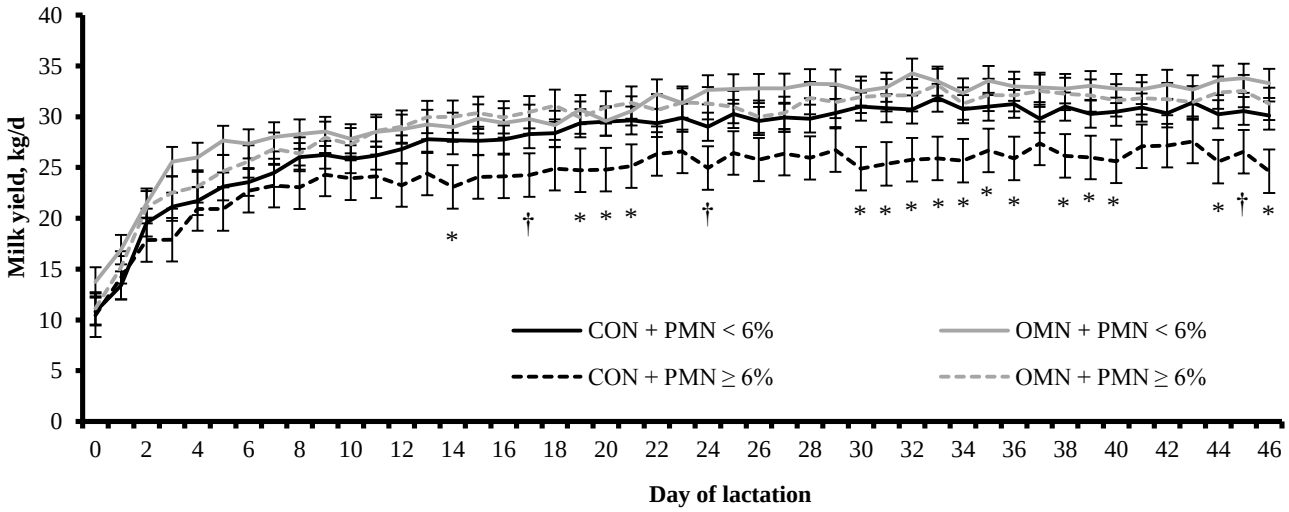

Figure 2. Serum concentrations of tumor necrosis factor alpha (**TNFα; Panel A**) and haptoglobin (**Panel B**) in dairy cows supplemented with Omnigen-AF (**OMN**; n = 19) or not (**CON**; n = 19) and administered an intravenous bolus dose of lipopolysaccharide (**LPS**) at 0 h. A treatment × hour interaction was detected for serum TNFα ($P = 0.03$), whereas a treatment effect was detected for serum haptoglobin concentrations ($P = 0.04$). Within hours, * = $P \leq 0.05$, † = $P \leq 0.10$

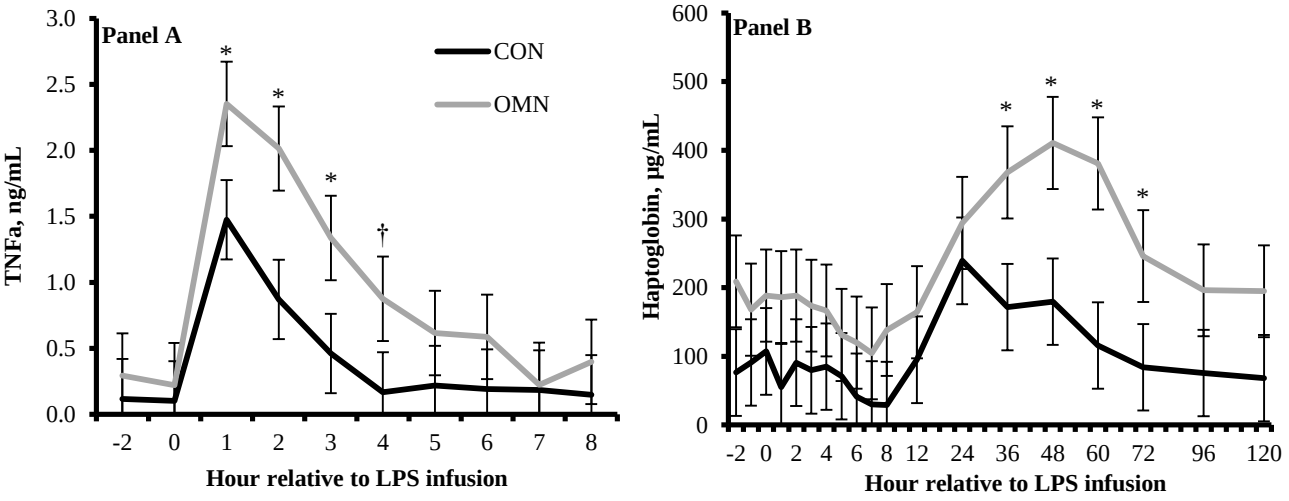

Table 3. Milk production parameters of dairy cows supplemented with Omnigen-AF (**OMN**; n = 19) or not (**CON**; n = 19) prior to and for 46 d after calving.[1]

Item	OMN	CON	SEM	*P* =
Milk yield, kg/d	30.3	27.1	0.9	0.02
Milk total solids, %	14.4	15.0	0.3	0.12
12% total solids-corrected milk, kg/d	36.1	33.3	1.2	0.07
SCC, cells/ml	326	450	114	0.45

[1] Individual milk production was recorded daily until d 46 of lactation. Values reported are LSM according to main treatment effects, given that no treatment × day interactions were detected ($P \geq 0.64$).

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Effects of maternal nutritional status on nutrient transporter expression in bovine utero-placental tissue on days 16 to 50 of gestation

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ABSTRACT: We hypothesized that maternal nutrition and day of gestation would impact mRNA expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2*, and *CAT-3* in beef heifers. Crossbred Angus heifers (n = 49) were synchronized, bred via AI, assigned to nutritional treatment (CON = 100% of requirements for 0.45 kg/d gain and RES = 60% of CON) and ovariohysterectomized on d 16, 34, or 50 of gestation (n = 6 to 9/d); non-pregnant (NP) controls were not bred and ovariohysterectomized on d 16 of the synchronized estrous cycle (n = 6). The resulting arrangement of treatments was a 2 × 3 factorial + 1. Caruncle (CAR), intercaruncular endometrium (ICAR), and fetal membranes (FM), were obtained from the pregnant uterine horn immediately following ovariohysterectomy. For NP controls, only CAR and ICAR were obtained. Relative expression of the glucose transporter *GLUT1* and cationic amino acid transporters *CAT-1*, *CAT-2*, and *CAT-3* was determined for each tissue utilizing NP-CAR and NP-ICAR tissue as the baseline. For FM, NP endometrium served as the baseline. There was no interaction of day and treatment in FM for any genes ($P \geq 0.05$). Expression of *GLUT1* and *CAT-1* both showed a day effect, being greater ($P < 0.05$) in FM on d 34 and 50, compared with d 16. In CAR there was no day × treatment interaction and *CAT-3* expression tended ($P = 0.06$) to be greater in CON vs. RES heifers. Additionally, expression of *GLUT1*, *CAT-1*, and *CAT-2* in CAR were greater ($P < 0.01$) on d 16 compared with d 34 and 50, d 34 compared to d 50, and d 16 and 34 compared with d 50, respectively. In ICAR, *CAT-2* showed a day × treatment interaction, being greater ($P = 0.01$) on d 50 CON compared with all other groups. Transporter *CAT-3* tended ($P = 0.09$) to be greater in day × treatment in ICAR on d 16 CON compared with all other days and treatments. The expression of *GLUT1* was greater ($P < 0.01$) in ICAR on d 16 than all other days. Arginine transporter *CAT-1* was greater ($P < 0.01$) in ICAR on d 34 and 50 compared with d 16. These results partially support our hypothesis and indicate that day was a more influential factor for mRNA expression of utero-placental glucose and cationic amino acid transporters than maternal nutritional status in heifers during early pregnancy.

Key words: arginine, gestation, glucose, maternal nutrition, transporters

INTRODUCTION

Fetal growth is vulnerable to maternal dietary nutrient deficiencies during the 1st trimester of gestation (Wu et al., 2004). During the first 50 days of gestation, organogenesis is taking place. This time period of gestation is a critical developmental window with significant cellular and tissue differentiation. Nutritional influences may alter the mammalian phenotype through affecting gene regulatory mechanisms involved in DNA synthesis and replication, thus "imprinting" potential susceptibilities to chronic disease and metabolic issues into the genome (Waterland and Jirtle, 2004). Currently, fetal undernutrition occurs in grazing livestock worldwide (Wu et al., 2004). Maternal undernutrition has been implicated in fetal growth restriction and altered placental growth, reduced amino acid and glucose transport, and increased apoptosis and autophagy, which overall can yield decreased fetal growth during gestation (Zhang et al., 2015). Before the establishment of hemotrophic nutrition, the placenta is developing and the fetus begins to utilize increasing quantities of glucose and amino acids (Groebner et al., 2011). Thus, the expression of glucose and amino acid transporters in the utero-placenta becomes essential to the viability of the conceptus. Therefore, we studied the utero-placental glucose transporter *GLUT1* (*SLC2A1*), which is present in most tissues throughout the body and is ubiquitous across species. The amino acid transporters investigated are *CAT-1*, *CAT-2*, and *CAT-3* (*SLC7A1*, *SLC7A2*, and *SLC7A3)* whose substrates are cationic amino acids such as arginine and lysine, which are directly linked to angiogenesis and cell proliferation. In this experiment, we tested the hypothesis that mRNA for glucose and cationic amino acid transporters in utero-placental tissues would be differentially expressed due to day of gestation and maternal nutritional status.

MATERIALS AND METHODS

Animals

Protocols described herein were approved by the North Dakota State University IACUC. Crossbred Angus heifers (n = 49, ~15 mo of age; average initial BW = 324.9 kg) were exposed to the 5-d CO-Synch + CIDR estrus synchronization protocol. Six heifers were not inseminated to serve as non-pregnant (**NP**) controls, but received ovariohysterectomy for tissue collections on d 16 of the synchronized estrous cycle.

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2. Appreciation is expressed to personnel at the NDSU Animal Nutrition and Physiology Center, NDSU Animal Science Nutrition Laboratory, Animal Science Reproductive Physiology Laboratory, and Animal Science Graduate Students Jordan Hieber and Evan Knutson for their assistance in completing this project.

Table 1. Primer Sets used for real-time quantitative reverse-transcription PCR

Gene[1]	Forward primer (5'-3')	Reverse Primer (5'-3')	Accession No.
GLUT1	CGGCTGCCCTGGATGTC	GCCTGGGCCCACTTCAAA	NM_174602
CAT-1	CCGATAATCGCCACCTTAACCT	ACCAGGTCCTTCAGGTCGAA	DQ399522
CAT-2	CTGCAAGTGCCAGGGACCCAC	GGTTGCAGCCCCAGCCAAAGT	XM_865568.3
CAT-3	GTAGCCCCAACCCAACTCGGC	TGCTAGGAAGGATCGAGGAGCTGT	NM_001078019.1
B-Actin	TGTCCACCTTCCAGCAGATGT	AGCTCAGTAACAGTCCGCCTAGA	AB098974.1

[1]*GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2*, and *CAT-3*- Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3. B-Actin- Reference gene used in all tissues to complete ΔΔ Ct method.

The remaining heifers (n = 6 to 9/d of gestation/treatment) were bred by AI bred to a common sire at 12 h after observed estrus and ovariohysterectomized at d 16, 34, or 50 of gestation.

Diet/Housing

Heifers were housed at the NDSU Animal Nutrition and Physiology Center. Heifers were acclimated to individual bunk feeding (American Calan, Northwood, NH) for two weeks before the beginning of the trial. Immediately following AI, heifers were randomly assigned to one of two treatment groups. Control heifers (**CON)** received 100% of NRC (2000) requirements for 0.45 kg/d gain to reach 80% of mature BW at first calving. Restricted heifers (**RES)** were placed on a 40% global nutrient restriction which was accomplished by reducing total diet delivery 60% of the control delivery. The diet was delivered via TMR and consisted of grass hay (73.4% NDF, 8.0% CP), corn silage (55.6% NDF, 6.3% CP), alfalfa haylage, (48.9% NDF, 16.4% CP), grain supplement, (32.6% NDF, 24.1% CP), and dried distillers grains (53.4% NDF, 31.3% CP), on a DM basis.

Sample Collection

Immediately following ovariohysterectomy (McLean et al., 2016), utero-placental tissues (caruncle, **CAR**; intercaruncular endometrium, **ICAR**; fetal membrane [chorioallantois], **FM**; cotyledon, **COT**; intercotyledonary placenta **ICOT**; and amnion, **AMN**) were obtained from the uterine horn containing the conceptus, as previously described (Grazul-Bilska et al., 2010, 2011). Fetal membranes were also collected only from heifers that were bred due to a lack of FM in NP controls. On d 50 of gestation, FM was split into COT and ICOT. Amnion was only collected on d 50. Once collected, all tissues were frozen in liquid nitrogen-cooled isopentane and stored at -80°C.

Real-Time Reverse Transcription Quantitative PCR (qPCR)

The RNA was extracted and purified using Aurum Total RNA Mini Kit (Bio-Rad, Hercules, CA), and cDNA was synthesized utilizing iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Total quantity of RNA was determined using Take3 module of a Synergy H1 Microplate Reader (BioTek, Winooski, VT). Optimal cDNA dilutions were determined by primer validation for each gene and tissue type across days of gestation. Primers were sourced through GenBank (Bethesda, MD; Table 1). Dilutions of 1:100 were utilized for *GLUT1* and *CAT-1*, and 1:10 for *CAT-2*, and *CAT-3* (Table 1). Gene expression was quantified using a 7500 Fast, Real-Time PCR System (Applied Biosystems, Grand Island, NY) with SYBR Green Master Mix (Bio-Rad Laboratories, Hercules, CA); 15 µL total for all genes. Relative expression was calculated using the ΔΔCt method (Livak and Schmittgen, 2001) with β-Actin, served as a reference gene.

Statistical Analysis

On d 50, COT, ICOT, and AMN mRNA level of expression were averaged within heifer to yield the FM value to complete the 2 × 3 factorial arrangement. Data were analyzed using the GLM procedure of SAS (SAS Inst. Inc. Cary, NY) with day, treatment and day × treatment in the model. If no significant interactions were present, main effects of maternal nutrition and day of gestation were reported. For COT, ICOT, and AMN on d 50 a comparison of tissue was conducted using the GLM procedure of SAS with individual heifer serving as the experimental unit. Means were separated using the LSMEANS procedure of SAS, and *P*-values ≤ 0.05 were considered significant. For CAR and ICAR, analysis of the 2 × 3 factorial arrangement was conducted using the GLM procedure of SAS (SAS Inst. Inc., Cary, NY) with day, treatment, and day × treatment in the model. Individual heifer served as the experimental unit. Means were separated using the LSMEANS procedure of SAS, and *P*-values ≤ 0.05 were considered significant. Additionally, contrasts were conducted comparing the mRNA expression for NP vs. pregnant heifer, d 16 vs. d 34

Table 2. Relative expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2*, and *CAT-3* in AMN, COT, and ICOT on d 50 of gestation using NP endometrium as a baseline value set to 1.

Gene[1]	AMN[2]	COT[3]	ICOT[4]	SEM[5]	*P*-value[6]
GLUT1	0.67[a]	0.24[b]	0.29[b]	0.07	< 0.01
CAT-1	0.30[a]	0.22[b]	0.17[b]	0.03	0.02
CAT-2	3.27[a]	1.42[ab]	0.82[b]	0.66	0.05
CAT-3	7.64[a]	0.73[b]	2.75[b]	1.38	< 0.01

[1]Gene = *GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2*, and *CAT-3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3.
[2]Amnion taken on d 50 of gestation.
[3]Cotyledons taken from fetal membranes on d 50 of gestation.
[4]Intercotyledonary tissue (fetal membrane tissue not including cotyledons; taken from fetal membranes on d 50 of gestation).
[5]Average SEM was used within gene; AMN n = 11, COT n = 14, ICOT n =14
[6]Probability values for the effect of tissue on level of expression of individual genes.
[a-b]Means within gene without a common superscript differ by tissue ($P \leq 0.05$).

Table 3. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2*, and *CAT-3* in fetal membranes (FM) due to CON and RES dietary treatments from d 16 to 50 of gestation in beef heifers using NP endometrium as a baseline value set to 1.

			Day of Gestation[2]					*P* – values[3]		
Gene[1]	Trt[4]		16	34	50	Trt[5]	SEM[6]	Day	Trt	Day × Trt
GLUT1	CON		0.11	0.25	0.38	0.25	0.08	0.04	0.70	0.90
	RES		0.19	0.27	0.38	0.28				
		Day[7]	0.15[b]	0.26[ab]	0.38[a]					
CAT-1	CON		0.04	0.22	0.22	0.16	0.17	< 0.01	0.70	0.99
	RES		0.05	0.24	0.23	0.17				
		Day	0.05[b]	0.23[a]	0.22[a]					
CAT-2	CON		0.42	0.84	1.97	1.08	0.66	0.09	0.87	0.82
	RES		0.24	1.16	1.57	0.99				
		Day	0.33	1.00	1.77					
CAT-3	CON		0.08	3.94	5.20	3.07	2.21	0.39	0.61	0.57
	RES		2.38	0.93	3.02	2.11				
		Day	1.23	2.43	4.11					

1Gene = *GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2*, and *CAT-3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3.
[2]Day of Gestation = number of days after AI. Each gene of interest expression value is reported as a fold change in relation to NP endometrium level of gene expression.
[3]Probability values for the effect of day, treatment, and day × treatment on the level of expression of individual genes.
[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 0.45 kg daily. RES = Heifers restricted to 60% of CON diet.
[5]Mean gene expression of treatment group across day of gestation within tissue and gene of interest.
[6]Average SEM used within gene. d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7
[7]Mean gene expression across treatment within day and gene of interest.
[a-b]Means within row lacking common superscript differ ($P \leq 0.05$).

Table 4. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2*, and *CAT-3* in caruncular CAR tissue due to CON and RES dietary treatments from d 16 to 50 of gestation and in non-pregnant (NP) controls set to 1.

			Day of Gestation[2]					*P* - values[3]					
Gene[1]	Trt[4]		16	34	50	Trt[5]	SEM[6]	NP vs. Preg	16 vs. 34 and 50	34 vs. 50	Day	Trt	Day × Trt
GLUT1	CON		2.40	0.93	1.38	1.57	0.44	0.21	< 0.01	0.47	< 0.01	0.77	0.23
	RES		3.38	0.76	0.89	1.67							
		Day[7]	2.89[g]	0.85[h]	1.14[h]								
CAT-1	CON		1.08	5.24	0.53	2.28	1.03	0.21	0.09	< 0.01	< 0.01	0.78	0.90
	RES		1.85	5.20	0.49	2.51							
		Day	1.47[h]	5.22[g]	0.51[h]								
CAT-2	CON		5.76	14.37	7.98	9.37	3.63	0.06	0.02	0.04	0.02	0.77	0.89
	RES		2.95	14.97	7.58	8.50							
		Day	4.36[h]	14.67[g]	7.78[gh]								
CAT-3	CON		1.29	2.23	4.28	2.60	1.09	0.44	0.24	0.11	0.20	0.06	0.90
	RES		0.45	0.99	2.05	1.16							
		Day	0.87	1.61	3.16								

[1]Gene = *GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2*, and *CAT-3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3.
[2]Day of Gestation = number of days after insemination. Each gene expression is given as a fold change in relation to NP level of expression set to 1.
[3]Probability values for effect of d, treatment, and day × treatment on level of expression of individual genes. Probability values for the contrast of mRNA level of expression of NP vs. Preg (all days of gestation), d 16 of gestation vs. d 34 and 50 of gestation, and d 34 vs. d 50 of gestation.
[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 0.45 kg daily. RES = Heifers restricted to 60% of CON diet
[5]Mean level of expression of treatment group across day of gestation within tissue and gene of interest.
[6]Average SEM was used within gene. NP n = 6, d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7
[7]Mean level of expression across treatment within day and gene of interest.
[a-c]Means within gene and tissue without a common superscript differ in day × treatment ($P \leq 0.05$).
[g-h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).

and 50 (pre-implantation vs. post-implantation), and d 34 vs. d 50 using the GLM procedure of SAS, with *P*-values ≤ 0.05 being considered significant.

RESULTS

COT, ICOT, and AMN d 50 Tissue Comparison

Glucose transporter *GLUT1* was greater ($P < 0.01$) in AMN compared to COT and ICOT (0.67 vs. 0.24 and 0.29 respectively; Table 2). Arginine transporter *CAT-1* was greater ($P = 0.02$) in AMN when compared to both COT and ICOT (0.30 vs. 0.22 and 0.17, respectively; Table 2). Cationic amino acid transporter *CAT-2* was greater ($P = 0.05$) in AMN compared to ICOT (3.27 vs. 0.82, respectively). The level of expression of *CAT-3* was greater ($P < 0.01$) in AMN compared to COT and ICOT (7.64 vs. 0.73 and 2.75, respectively).

Fetal Membranes (FM)

There was no day × treatment interaction or main effect of treatment for any gene in FM ($P > 0.05$). Expression of *GLUT1* was greater ($P = 0.04$) on d 50 of gestation compared with d 16 (0.38 vs. 0.15, respectively; Table 3). Cationic amino acid transporter *CAT-1* expression was greater ($P < 0.01$) on d 34 and 50 compared to d 16 (0.23 and 0.22 vs. 0.05, respectively; Table 3). The mRNA expression of *CAT-2* tended to be greater ($P = 0.09$) on d 50 of gestation compared with d 16.

Maternal Caruncules (CAR)

There was no day × treatment interaction ($P \geq 0.05$) on the mRNA expression of *GLUT1*, *CAT-1*, *CAT-2*, or *CAT-3* in CAR. Expression of *CAT-3* showed a tendency ($P = 0.06$) to be greater across day of gestation in CON vs. RES (2.60 vs. 1.16; Table 4). Expression of *GLUT1* was greater ($P < 0.01$) on d 16 of gestation compared to d 34 and 50 (2.89 vs. 0.85 and 1.14 respectively). The mRNA expression of *CAT-1* was greater ($P < 0.01$) on d 34 compared to d 16 and 50 (5.22 vs. 1.47 and 0.51 respectively; Table 4). Additionally, mRNA expression of *CAT-1* tended to be greater ($P = 0.09$) in the post-implantation (d 34 and 50) vs. implantation (d 16). Expression of cationic amino acid transporter *CAT-2* was greater ($P = 0.02$) on d 34 compared to d 16 of gestation (14.67 vs. 4.36, respectively). In addition, *CAT-2* mRNA expression showed a tendency ($P = 0.06$) to be greater in pregnant vs. NP heifers.

Maternal Intercaruncular Endometrium (ICAR)

The expression of *CAT-2* showed a day × treatment interaction ($P = 0.01$), being greater with d 50 CON heifers having greater expression compared with d 16 and 50 RES and d 34 CON heifers(Table 5). The cationic amino acid transporter *CAT-3* tended ($P = 0.09$) to be greater in d 16 CON compared with all other days and treatments. The mRNA expression of *GLUT1* was greater ($P < 0.01$) on d 16 of gestation compared to d 34 (2.11 vs. 0.75). Arginine and Lysine transporter *CAT-1* was greater ($P < 0.01$) on d 34 and 50 compared to d 16 (14.62 and 11.13 vs. 0.58, respectively). Additionally, *CAT-1* mRNA expression was greater in ICAR ($P < 0.01$) in pregnant compared to NP heifers (8.78 vs. 1.00, respectively).

DISCUSSION

Fertilization rates for first service AI are approximately 90% in beef heifers (Bridges et al., 2013); however, by d 30 of gestation, only 50 to 60% of heifers maintain a viable pregnancy. Moreover, Thatcher et al., (1994) indicated that up to 40% of all embryonic loss occurs before d 40 of gestation in sheep and cattle. Glucose and amino acids, specifically arginine, are crucial for proper energy metabolism and growth, and are key regulators of mTOR, which is linked to angiogenesis and cell proliferation, causing increased fetal growth and mitigating apoptosis (Tan and Miyamoto, 2016). These data are the first report of the impacts of maternal nutritional treatment and day of gestation on the mRNA expression of glucose and cationic amino acid transporters *GLUT1*, *CAT-1*, *CAT-2*, and *CAT-3* in bovine utero-placental tissues on d 16 to 50 of gestation. The expression of all transporters investigated was greatest on d 50 in AMN compared to COT and ICOT. Amniotic fluid contains the nutrient reserve from which the conceptus draws to meet its energetic and growth requirements prior to transplacental exchange. The reported data further demonstrate the increased emphasis on transport of nutrients across the AMN to provide nutrients to the conceptus. Data presented herein for effects of day of gestation on nutrient transporter expression are similar to those previously reported (Crouse et al., 2015). Before the establishment of transplacental exchange, nutrient transporters are the only method of supplying nutrients to the conceptus. Therefore, it is of interest to evaluate the concentration of nutrients in the maternal and fetal fluids (serum, histotroph, and allantoic and amniotic fluids) to determine whether nutrient restriction during early gestation affects nutrient concentrations in maternal and fetal fluids or nutrient transport capacity, thereby altering abundance of nutrients available for transport to the conceptus.

IMPLICATIONS

We interpret these data to imply that a 40% global maternal nutritional restriction may affect the mRNA expression of some (*CAT-3*), but not all utero-placental nutrient transporters investigated herein. The effects of day of gestation on the mRNA expression of glucose and cationic amino acid transporters reflects the changing nutrient supply and demand curve necessary for proper conceptus growth. The surgical techniques employed for collection of tissues and fluids from the bovine utero-placenta allow for a detailed assessment of the multiple mechanisms at play that support fetal growth and development during early gestation. Moreover, additional knowledge in this area will facilitate increased efficiencies of beef cattle production and contribute to meeting the projected world food demands.

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Table 5. Level of expression of nutrient transporters *GLUT1*, *CAT-1*, *CAT-2*, and *CAT-3* in intercaruncular ICAR tissue due to CON and RES dietary treatments from d 16 to 50 of gestation and in non-pregnant (NP) controls set to 1.

			Day of Gestation[2]					*P* - values[3]					
Gene[1]	Trt[4]		16	34	50	Trt[5]	SEM[6]	NP vs. Preg	16 vs. 34 and 50	34 vs. 50	Day	Trt	Day × Trt
GLUT1	CON		2.44	0.63	1.77	1.61	0.39	0.43	< 0.01	0.10	< 0.01	0.26	0.42
	RES		1.77	0.87	1.08	1.24							
		Day[7]	2.11^{g}	0.75^{h}	1.43^{gh}								
CAT-1	CON		0.65	13.86	9.94	8.15	2.59	< 0.01	< 0.01	0.13	< 0.01	0.56	0.89
	RES		0.51	15.37	12.33	9.41							
		Day	0.58^{h}	14.62^{g}	11.13^{g}								
CAT-2	CON		6.83^{ab}	2.31^{c}	7.78^{a}	5.64	1.43	0.04	0.88	0.56	0.22	0.68	0.01
	RES		3.10^{bc}	6.08^{abc}	3.17^{bc}	4.11							
		Day	4.97	4.19	5.48								
CAT-3	CON		9.69	1.55	5.53	5.59	1.89	0.09	0.12	0.27	0.13	0.45	0.09
	RES		3.87	4.25	5.05	4.39							
		Day	6.78	2.90	5.29								

[1]Gene = *GLUT1*- Glucose transporter solute carrier family 2 member 1. *CAT-1*, *CAT-2*, and *CAT-3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2, and 3.

[2]Day of Gestation = number of days after insemination. Each gene expression is given as a fold change in relation to NP level of expression set to 1.

[3]Probability values for effect of d, treatment, and day × treatment on level of expression of individual genes. Probability values for the contrast of mRNA level of expression of NP vs. Preg (all days of gestation), d 16 of gestation vs. d 34 and 50 of gestation, and d 34 vs. d 50 of gestation.

[4]CON = Heifers fed a diet that meets 100% of NRC requirements to gain 0.45 kg daily. RES = Heifers restricted to 60% of CON diet.

[5]Mean level of expression of treatment group across day of gestation within tissue and gene of interest.

[6]Average SEM was used within gene. NP n = 6, d 16 CON n = 7, d 16 RES n = 7, d 34 CON n = 6, d 34 RES n = 9, d 50 CON n = 7, d 50 RES n = 7

[7]Mean level of expression across treatment within day and gene of interest.

[a-c]Means within gene and tissue without a common superscript differ in day × treatment ($P \leq 0.05$).

[g-h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).

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Key metabolic pathways associated with differences in weight maintenance and gain in mature cow skeletal and adipose tissue

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ABSTRACT: During the production year of a cow, the majority of nutrients are used to support maintenance. Differences in feedstuff utilization and metabolism can impact the ability of the cow to meet maintenance requirements. Tissue specific metabolism is critical to energy homeostasis of the animal, and therefore regulation of metabolism is critical to understand. The objective of this research was to determine whether cows that differ in efficiency of weight maintenance and weight gain differ in the relative abundance of transcripts associated with protein and lipid turnover of skeletal muscle and adipose tissue, respectively. Crossbred cows (n = 121) were feed restricted for 112 d followed by an *ad libitum* feeding period for 98 d. Individual feed intake was monitored and body weights were collected to estimate average daily gain (ADG). Adipose and muscle biopsies were collected at d 105 of restricted feeding and at d 49 of *ad libitum* feeding. Total RNA was extracted from these tissues of the cows with the highest (n = 6) and the lowest (n = 6) ADG during the *ad libitum* period. The Affymetrix GeneAtlas microarray system was used to determine relative transcript abundance differences between ADG classes within feeding periods and tissue type. Subsequent analyses using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) and Ingenuity Pathway Analysis (IPA) programs identified key gene clusters and pathways associated with differential gene expression, largely including pathways associated with lipid and carbohydrate metabolism, cell-cell signaling and interaction, and cellular function and maintenance. These data suggest key metabolic pathways may be critical to differences in weight maintenance and gain.

Key words: adipose tissue, metabolism, skeletal muscle

[1]The USDA is an equal opportunity provider and employer.

INTRODUCTION

During the production year the cow uses nutrients to support conceptus growth, milk production, work (grazing and locomotion), and maintenance. The majority of the nutrients are used to support maintenance. Substrate cycling has been identified as one of the major contributors toward energy expenditure associated with maintenance in mature cows. Caton and Dhuyvetter (1996) reported that maintenance can account for 60-90% of total herd energy expenditures. Specifically, muscle and adipose tissue account for 27% of total energy use (Caton et al., 2000).

Muscle and adipose tissue are major energy sinks because of their roles in energy storage, use, and release. Additionally, these tissues are critical to growth components and thus product formation. It has been suggested that more efficient animals have decreased fat deposition with increased muscle mass (Richardson and Herd, 2004). Fat deposition is more energetically costly than muscle deposition and could explain why low efficiency animals tend to be less lean. Additionally, it has been suggested that protein turnover is greater in low efficiency versus high efficiency cattle, indicating increased lean muscle deposition (Oddy et. al. 1995, 1998; Herd and Arthur, 2009).

These data support further investigation into specific mechanisms of these active tissues and the potential influence on overall metabolic efficiency of the animal. Understanding efficiency and investigating ways to improve efficiency in the herd is critical to beef production, yet the specific mechanisms associated with these differences remain unclear. Because of the roles that muscle and adipose tissue play in energy use as well as growth, we hypothesized that these two tissues and their activity may contribute to differences in efficiency. Our objective was to determine if cows that differ in efficiency of weight maintenance and weight gain differ in the relative abundance of transcripts for genes associated with pathways regulating metabolism and function of skeletal and adipose tissue.

MATERIALS AND METHODS

The U.S. Meat Animal Research Center (**USMARC**) Animal Care and Use Committee reviewed and approved all animal procedures. The procedures for handling cattle complied with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Animal Management and Diets

Cross-bred cows (n =121) originating from sampling sires used in the industry were utilized for this study. Angus, Hereford, and MARC III composite (¼ Angus, ¼ Hereford, ¼ Pinzgauer, ¼ Red Poll) cows were bred by artificial insemination to Angus, Hereford, Simmental, Limousin, Charolais, Gelbvieh and Red Angus bulls. The F_1 bulls from these matings that had Angus and Hereford dams were mated to F_1 cows from these matings in multiple-sire pastures to produce 2-, 3-, and 4-breed cross

progeny. The resulting female progeny were kept and raised to have their first calf as 2-year-olds. At 5 years of age cows were not bred, and were moved to an individual feed intake facility equipped with Calan Gates (American Calan, Northwood, NH) the week after their calves were weaned. Cows were fed a ration that contained as a percent of DM 27.0 ground alfalfa hay, 5.0 corn, 67.3 corn silage, and 0.2 salt. Cows were weighed on two consecutive days 21 d after weaning, and BW was averaged. Feed offered was designed to provide 120 kcal ME/kg $BW^{0.75}$. Cows were fed the same amount of feed for 112 d. At 112 d, cows had *ad libitum* access to the same ration for an additional 98 d. Cows were fed once a day and feed refusals were measured weekly. During the feed restriction cows were weighed on d 0, 1, 14, 28, 56, 84, 111, and 112. During the *ad libitum* period cows were weighed on d 0, 14, 28, 42, 56, 70, 84, 97, and 98. Individual BW was regressed on time during the *ad libitum* period using a quadratic equation, and final BW gain was calculated from the regression equation.

Tissue Samples

Adipose and muscle biopsies were performed on all cows 105 d after the start of the feed restriction started, and 49 d after the start of the *ad libitum* feeding period. The cows with the highest gain (**H Gain**; n = 6; mean ADG = 1.987 ± 0.11 kg/d) and the lowest gain (**L Gain**; n = 6; mean ADG = 0.983 ± 0.14 kg/d) during the *ad libitum* period were selected for adipose and muscle tissue microarray analyses. This resulted in a total of 48 samples for microarray analysis (12 cows; 2 tissue types; 2 sampling times).

The sample site was scrubbed with betadine and rinsed. Twenty milliliters of lidocaine was injected subcutaneously at the incision site, and a 5-cm incision was made through the skin with a scalpel above the fat pad between the hooks and pins (lumbo-sacral fat pad). A 50-100 mg adipose tissue sample was harvested with iris scissors. The sample was immediately frozen in liquid nitrogen and stored at -80° C. Using the same incision site, a 50-100 mg *gluteus medius* muscle biopsy was taken with a U.C.H. skeletal muscle biopsy needle. The sample was immediately frozen in liquid nitrogen and stored at -80° C. The wound was closed with Braunamid 4 USP (B. Braun AESCULAP, Germany) suture, which was then removed 14 d later. The sample collected during restricted feeding was from the left side of the cow, and the sample collected during *ad libitum* feeding was from the right side.

RNA Extraction and Quality Assessment

Total RNA was extracted from 50-100 mg of tissue by homogenization with TriPure reagent (Roche, Indianapolis, IN) following the manufacturer's protocol with the exception of an increased time of centrifugation from 15 to 20 min following the addition of chloroform. RNA pellets were resuspended in sterile water and RNA was stored at -80° C until further processing. Purified RNA was quantified using a NanoDrop 8000 spectrophotometer (Thermo Scientific, Wilmington, DE). The quality of total RNA was determined by running samples on a RNA 6000 LabChip kit (Agilent Technologies, Waldbronn, Germany) with the Agilent 2100 bioanalyzer. Additionally, RNA electrophoresis was utilized to determine quality based on intensity of the 28S and 18S RNA. Absorbance was measured by spectrophotometry at 260 nm. RNA quality was additionally assessed with the 2100 Bioanalyzer (Agilent, Santa Clara, CA).

Affymetrix GeneAtlas Microarray

To evaluate the differential expression of genes in adipose and muscle tissues, the Affymetrix GeneAtlas System (Santa Clara, CA) was used in conjunction with Bovine 1.1ST array strip, as described by Lindholm-Perry et al. (2016). The Bovine Gene 1.1ST Array strip represents 24,341 genes and 526,810 probes (23 probes/gene). Briefly, 250 ng of total RNA with the Poly-A RNA controls (5 µL) was ultimately converted to single stranded cDNA followed by fragmentation and TdT labeling using the WT Expression Kit manufacturer's instructions (Ambion®, Life Technologies Corporation). The fragmented and labeled single stranded cDNA (23 ng/µL) was then hybridized on the Bovine Gene 1.1ST Array strip overnight (20 h at 45° C), using Affymetrix HybWashStain Kit and Affymetrix standard protocols. The fluidics protocol used was for the GeneAtlas Fluidic Station and Ge4neAtlas Instrument Control software. Imaging was completed using the GeneAtlas Imaging Station. The Affymetrix Expression software converted CEL formatted files to CHP files (Colombo et al., 2014). Both Guanine Cytosine Count Normalization (**GCCN**) and Signal Space Transformation (**SST**) algorithms were applied. Samples were further processed and normalized using the Robust Multichip Analysis (RMA; Irizarry et al., 2003). These files were then used to identify differentially expressed genes (**DEG**).

Data Analysis

The database for annotation, visualization, and integrated discovery (**DAVID**) v6.7 was used to assess the biological function of DEG. Functional annotation classification was based on a set of novel fuzzy clustering techniques and grouped functionally related genes on the basis of annotation term co-occurrence (Huang et al., 2009). The Kyoto Encyclopedia of Genes and Genomes (**KEGG**) Pathways for these genes provided biological interpretation based on a network of genes.

The Ingenuity ® Pathway Analysis (**IPA**®, QIAGEN Redwood City, www.qiagen.com/ingenuity) tool depicted gene interactions on a system level. Results of this software are based on human-interpretation of peer-reviewed gene and protein interaction literature. The Top Canonical Pathways and Primary Molecular and Ceullular Functions generated by IPA were utilized to analyze 4 comparisons; 1) Feed-restricted Adipose H Gain vs. L Gain, 2) Feed-restricted Muscle H Gain vs. L Gain, 3) *Ad libitum* Muscle H Gain vs. L Gain, 4) *Ad libitum* Adipose H Gain v. L Gain. The *p*-value provided for the canonical pathways was

determined by using the fit of the significant genes and the size of the network.

RESULTS

Feed-restricted Adipose (H Gain vs. L Gain)

A total of 113 genes were differentially expressed in the feed-restricted adipose tissue across the H Gain and L Gain cows. The transcription was increased for 45 genes, and decreased for 68 genes. Thirteen of the genes were not annotated. There were 3 KEGG pathways over-represented in this comparison including sphingolipid metabolism, pyruvate metabolism, and glycerophospholipid metabolism. The top canonical pathways were acetate conversion to acetyl-CoA, oxidative ethanol degradation III, cytidine diphosphate (CDP)-diacylglycerol biosynthesis I, glutathione redox reactions I, and ethanol degradation IV. The primary molecular and cellular functions associated with these DEG were lipid metabolism, molecular transport, small molecule biochemistry, cell-to-cell signaling and interaction, and cellular assembly and organization.

Feed-restricted Muscle (H Gain vs. L Gain)

Thirty-eight genes were differentially expressed in the feed-restricted muscle samples across the H Gain and L Gain cows. There were 25 genes with higher transcript abundance and 13 genes with lower transcript abundance. Of these, 19 genes with increased transcription and 2 genes with decreased transcription were annotated. There were no KEGG pathways over-represented for this comparison. The top canonical pathways were spermine biosynthesis, spermidine biosynthesis I, circadian rhythm signaling, ephrin A signaling, and semaphorin signaling in neurons. Lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism, carbohydrate metabolism, and cellular compromise were identified as the molecular and cellular functions associated with these DEG.

Ad libitum Muscle (H Gain vs. L Gain)

Twelve genes were differentially expressed when comparing the H Gain and L Gain *ad libitum* muscle tissue. Of these, 8 genes had increased transcription and 4 genes had decreased transcription. Only 3 of these genes were annotated: microRNA mir-2417 (*MIR2417*), with a 2.14 fold change in H Gain animals; FBJ murine osteosarcoma viral oncogene homolog B (*FOSB*), with a -2.6 fold change in H Gain animals; and coenzyme Q10 homolog B (*COQ10B*) with a fold change of -2.6 in H Gain animals. There were no KEGG pathways over-represented in this comparison. The top canonical pathway was cyclin-dependent kinase 5 (**CDK5**) signaling. The primary molecular and cellular functions associated with these DEG were gene expression, cell-to-cell signaling and interaction, cellular development, lipid metabolism, and molecular transport.

Ad libitum Adipose (H Gain vs. L Gain)

Sixty genes were differentially expressed between the H Gain and L Gain *ad libitum* muscle tissue; 33 genes had higher transcript abundance and 27 genes had lower transcript abundance. Six of the genes have not been annotated to-date. There were 3 KEGG pathways identified in this comparison including ECM-receptor interaction, focal adhesion, and systemic lupus erythematosus. The top canonical pathways were intrinsic prothrombin activation pathway, atherosclerosis signaling, glutamate removal from folates, coagulation system, and granulocyte adhesion and diapedesis. Cellular assembly and organization, cellular function and maintenance, cellular movement, cell-to-cell signaling and interaction, and cellular compromise were identified as the molecular and cellular functions.

DISCUSSION

The KEGG pathways that were over-represented indicated that the DEG from the adipose samples shifted from lipid and carbohydrate metabolism in the feed-restricted state to pathways associated with signaling molecules and interaction, cellular processes, and auto-immune disease during the *ad libitum* feeding period. The top canonical pathways generated from IPA reflected these results. Acetate conversion to acetyl-CoA and oxidative ethanol degradation pathways were down-regulated, indicating decreased energy production in the feed-restricted feeding period. The top canonical pathways during the *ad libitum* feeding periods were predominantly associated with immune response and cell injury. These data suggest that during a period of feed-restriction the primary pathways associated with the DEG reflect modulation of lipid and carbohydrate metabolism, in response to nutrient restriction. However, the pathways associated with adipose tissue during the *ad libitum* feeding period were associated with cellular processes, immune response, and cell injury. This may suggest that when ample nutrition is available the metabolic pathways shift away from macromolecule synthesis and degradation to pathways that regulate more specific cellular function.

While there were no KEGG pathways determined in the muscle tissue, the IPA results indicated that the top canonical pathways determined by the DEG of the muscle samples were primarily involved in signaling pathways. During the feed-restricted period, there was an up-regulation of polyamine synthesis and subsequent spermidine synthesis, both of which are critical for cell growth. Interestingly, during the *ad libitum* feeding period only one canonical pathway was determined, CDK5 signaling. Because CDK5 signaling is primarily associated with inhibiting down-stream biological processes, it is critical for cellular formation, maintenance, and function to regulate this signaling pathway.

Metabolic pathways are critical to maintenance of homeostasis. In this study, homeostasis was disrupted by a period of feed restriction followed by *ad libitum* feed. Additionally, the cows used in this study were divergent for ADG. This suggests that while homeostasis was disrupted, the two groups may respond to this disruption in different

ways, reflecting their differences in weight gain and efficiency. Many metabolic pathways are energetically expensive, and thus, regulation of these pathways is likely to play a role in whole animal efficiency. These include macromolecule turnover, transport processes, substrate cycling, detoxification, and synthesis, all of which are important for nutrient assimilation in the animal and are highly energetically expensive (Herd and Arthur 2009; Ferrell, 1988). These metabolic functions are necessary not only for animal maintenance but also for growth and meat production in beef cattle. Because these processes also require a high percentage of total energy, they may be influential on ultimate animal efficiency. Literature suggests other potential mechanisms specific to muscle and adipose tissue that may be contributing to difference in feed efficiency. These include processes of metabolism in terms of obtaining chemical energy from the consumed energy-rich nutrients, repartitioning of substrates to meet demands of specific tissues, and synthesis and degradation of biomolecules needed for cellular function (Nelson and Cox, 2005). Additional avenues of muscle and adipose function that may contribute to differences in efficiency may include the metabolic plasticity of these tissues and the impact that endocrinology specific to these tissues has on both anabolic and catabolic processes (Welch et al., 2012). A critical next step for this research is to validate these DEG determined to be of particular importance or interest in this study.

IMPLICATIONS

Improved efficiency of beef cows is critical to economic and production gains in a cattle operation. Because mature cows are one of the highest input sectors of the beef herd and feed prices remain the largest portion of operating costs, identifying underlining mechanisms of improved efficiency in mature cows is critical. It has been well established that muscle and adipose tissue are highly metabolically active and are also critical to weight maintenance and gain of an animal. A transcriptomic approach to identifying key differences between weight gain classes in these two tissue types may add to the expanding knowledge of underlying mechanisms potentially driving differences in efficiency and may enable future alterations or optimizations of metabolic pathways important to herd efficiency.

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Altered rumen microbial populations in response to high sulfate water in lambs

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ABSTRACT: Water is involved directly or indirectly in essentially every bodily process. Therefore, access to quality water sources is critical for livestock wellbeing. In the western U.S., however, high sulfate (SO_4^{2-}) water sources are frequently encountered. High SO_4^{2-} water can cause overproduction of ruminal H_2S and result in compromised health and performance of the host. An initial trial (Trial 1) was conducted to determine the impact of high SO_4^{2-} drinking water on the rumen microbiome of growing lambs. A follow-up trial (Trial 2) then sought to confirm rumen microbial species involved in the response to high SO_4^{2-} drinking water and additionally identify species that adapt to SO_4^{2-} challenges. Each trial consisted of individually penned Hampshire-cross lambs (n = 43 in Trial 1; n=16 in Trial 2) which had access to *ad libitum* feed and high SO_4^{2-} water (3,000 mg SO_4^{2-}/L) for a 28 d period. Trial 2 also included a 7 d post-treatment period to obtain recovery data for later analysis. DNA was extracted and sequenced from d 0, 7, and 28 rumen samples and then compared with known 16S rDNA reads for microbial identification. Operational taxonomic units (OTU) were defined as sequence clusters with ≥ 97% identity and analyzed for the fixed effect of sampling day using the GENMOD procedure of SAS. Trial 1 resulted in a total of 145 OTU found in at least 1 of the 24 sequenced samples (8 lambs; 3 sampling dates); 8 OTU were affected ($P \leq 0.05$) by sampling day. Trial 2 resulted in 287 OTU identified in at least 1 of the 24 sequenced samples (8 lambs; 3 sampling dates), with sampling day affecting ($P \leq 0.05$) 38 of those OTU. Collectively, these results indicate a shift in rumen microbe relative abundance in response to high SO_4^{2-} water. Abundance variation may confer differences in host animal ability to tolerate and adapt to high SO_4^{2-} water. Similarities in microbial abundance changes across the two trials suggest that particular species are especially reactive to high ruminal SO_4^{2-} and are likely important to host response. Furthermore, certain microbial species demonstrated greater potential to adapt over time to a high SO_4^{2-} environment. Greater understanding of the rumen microbes involved in the response to high SO_4^{2-} drinking water is necessary for development of effective treatment and prevention strategies for ruminant livestock maintained in high SO_4^{2-} water regions.

Key words: DNA sequencing, microbes, rumen, sheep, sulfate

INTRODUCTION

In the western U.S., livestock frequently only have access to water sources that are less than ideal due to a combination of competition with urbanization and mineral extraction (Raisbeck et al., 2007). Livestock drinking water is often high in sulfur (**S**), especially in the form of sulfate (**SO_4^{2-}**). Ruminants possess the unique ability to reduce dietary S and SO_4^{2-} via microbes within the rumen, and therefore drinking water sources high in either of these can greatly impact dietary S intake values (Nichols et al., 2012). Sulfur-reducing bacteria (e.g., *Desulfovibrio* and *Desulfotomaculum*) can utilize lactate and SO_4^{2-} as substrates to generate acetate and S^{2-}, which can further react with free H^+ to form H_2S where it accumulates in the rumen gas cap. The gas can be eructated and re-inhaled by the animal, causing neural damage and the onset of S-induced polioencephalomalacia (Gould et al., 2002). It has been proposed that the bacteria responsible for SO_4^{2-} reduction are capable of adapting to changing levels of dietary S and SO_4^{2-} (Cummings et al., 1995a). Variation in host animal tolerance to high dietary S has been demonstrated in beef cattle (Cammack et al., 2010). This variation is postulated to be due, in part, to differences in ruminal SO_4^{2-} reducing bacteria populations which are capable of adapting to changing levels of dietary S. Therefore, we hypothesized that the rumen microbiome of lambs will be altered in response to a high SO_4^{2-} drinking water treatment. Our objectives were to determine and confirm changes in abundance of rumen microbial species and identify microbial species that potentially adapt to a high SO_4^{2-} environment. A better understanding of the relationship between dietary SO_4^{2-} intake and individual rumen microbial species could facilitate the development of viable prevention and treatment methods to optimize livestock health and performance in regions with high SO_4^{2-} water.

MATERIALS AND METHODS

Animal Care. All procedures were approved by the University of Wyoming Animal Care and Use Committee. The initial lamb trial (Trial 1) occurred July 27th to August 23rd, 2013, at the University of Wyoming's Laramie Research Extension Center. The follow up trial (Trial 2) took place February 19th to March 26th, 2015, at the same location. Hampshire-cross growing lambs (n = 43; with initial BW 48.76 ± 16.44 kg in Trial 1; n = 12; with initial BW 75.52 ±

14.4kg in Trial 2) were randomly allotted to individual pens in a confinement facility for a 28 d feed and water intake trial. Trial 2 also included a 7 d post-treatment period to allow for collection of recovery data to be analyzed at a later time. Lambs were acclimated to the pelleted forage diet for 23 d prior to the start of Trial 1 and 20 d prior to Trial 2 to allow for adjustment to the diet as well as individual pens. All animals were administered the same forage-based pelleted diet (67.7% alfalfa and 27.5% wheat middlings; 16.2% CP, 36.3% NDF, 2.31 Mcal ME/kg, DM basis). Lambs were fed *ad libitum*, and orts were weighed back daily. On d 0, 7, and 28 2-d BW were collected and averaged.

Water Sulfate. Prior to the high SO_4^{2-} water administration, lambs were provided water from the research facility with 67 mg SO_4^{2-}/L. To create the desired level of 3,000 mg SO_4^{2-}/L for the high S water treatment, sodium sulfate (Na_2SO_4) was mixed daily with water from the research facility. The water mixture was tested daily during the first 2 wk of SO_4^{2-} administration at the Wyoming Department of Agriculture Analytical Services (Laramie), and then every other day for the remainder of the treatment period. Actual levels over the duration of the trial period averaged 3043.89 ± 746.11 mg SO_4^{2-} /L during Trial 1 and 3,000 ± 500 mg SO_4^{2-}/L for Trial 2. This level of SO_4^{2-} was chosen to avoid acute toxicity. Water was provided *ad libitum*; disappearance was measured twice daily to determine lamb water intake. Water evaporation was also monitored daily.

Animal Selection. For each trial, 8 lambs were selected for DNA sequencing based on individual health and performance data, including feed and water intake. No signs of sPEM were observed in any of the lambs.

Microbial DNA Sequencing. From the rumen fluid samples, DNA was extracted using methods detailed by Yu and Morrison (2004); 5 µg of 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 by truncating each read after the first run of 3 bases using a phred quality score < 15, quality-trimmed by omitting reads with < 85 base pairs or a quality score of < 25, and compared with a database of 27K known 16S rDNA genes using the Bowtie reference-based assembly tool (Johns Hopkins, Baltimore, MD). For the read sequence and the database sequence to match, they were required to have ≥ 97% likeness. Operational taxonomic units (OTU) were defined as sequence clusters with ≥ 97% identity; therefore, each OTU was assumed to be a single microbial species.

Statistical Analysis. The OTU data sets from each of the two trials were analyzed separately, as we aimed to confirm results from the initial trial. A generalized linear model was fitted using the GENMOD procedure of SAS to determine the effects of sampling day on OTU abundance assuming a Poisson distribution; $\alpha = 0.05$ was considered statistically significant. Raw *P*-values from the Poisson regression were corrected for multiple tests using the false-discovery rate correction of Benjamini and Hochberg (1995). Because means produced from the Poisson regression were non-normally distributed, treatment means were generated using the GLM procedure of SAS. Additionally, those OTU with a significant sampling day effect were further tested for linear and quadratic effects using orthogonal contrasts.

RESULTS AND DISCUSSION

Trial 1 resulted in a total of 145 OTU found in at least 1 of the 24 sequenced samples (8 lambs; 3 sampling dates). Taxa with ≤ 3 counts (n = 42) for any one sample were considered to be sequencing artifacts (or potentially false positives) and thus eliminated. Of the remaining taxa (n = 103), 8 OTU were affected ($P \leq 0.01$) by sampling day. Trial 2 resulted in 287 OTU identified in at least 1 of the 24 sequenced samples. After removal of sequencing artifacts, 167 OTU remained and sampling day affected ($P \leq 0.05$) the abundance of 23 of those remaining OTU (Table 2). There were five microbial species that were consistently affected ($P \leq 0.05$) by sampling day across the two trials: *Prevotella ruminicola, P. albensis, P. nigrescen, P. genomosp.,* and *Butyrivibrio fibrisolvens*.

In addition to being the primary genus effected by sampling day, *Prevotella* comprised the largest portion of the microbiome in all lambs throughout both trials. It has been well established that *Prevotella* is typically the most dominant genus in the rumen (Stevenson and Weimer, 2009). Of the individual *Prevotella* species, *P. ruminicola* had the greatest abundance across trials regardless of sampling day. A linear increase ($P = 0.004$) in abundance of *P. ruminicola* was observed over the course of Trial 1; however, a linear decrease ($P \leq 0.001$) in *P. ruminicola* abundance was observed during Trial 2. Van Soest (1994) defined *P. ruminicola* as a carbohydrate fermenter that is able to utilize cellulose, hemicellulose, and pectin as well as starches, proteins, and sugars (both simple and complex). As this species can utilize a wide variety of substrates, it tends to be a predominant species within the rumen (Carberry et al., 2012). *Prevotella albensis* abundance increased ($P = 0.004$) initially at d 7 during Trial 1, but did not increase ($P = 0.015$) until d 28 in Trial 2. There was a quadratic effect ($P \leq 0.004$ in Trial 1; $P = 0.014$ in Trial 2) observed in abundance of *P. nigrescen* during both Trials 1 and 2. The return to pre-treatment abundance levels by d 28 suggests that *P. nigrescen* may have the ability to adapt to a high SO_4^{2-} environment. Finally, an unknown *Prevotella* species, *P. genomosp.*, was affected ($P \leq 0.001$) by sampling day in both trials. However, while it appears that these species are responsive to a high SO_4^{2-} environment, it is unclear if these are indeed the same species of *Prevotella*.

Butyrivibrio fibrisolvens decreased linearly ($P = 0.001$ in Trial 1; $P = 0.020$ in Trial 2) over the two trial periods, to nearly non-present by d 28. While *B. fibrisolvens* can utilize a wide variety of substrates, it is primarily noted for its ability to degrade cellulose and produce butyrate as the major fermentation acid (Hespell et al., 1993). Altered abundance of fibrolytic bacteria, including *B. fibrisolvens* has been associated with S supplementation (McSweeny and

Denman, 2007). While *B. fibrisolvens* can be cultured, the capacity of this species to persist and maintain abundance in the rumen will require further research (Klieve et al., 2003).

No differences in S-reducing bacteria were apparent in this study. Cummings et al. (1995b) reported an increase in H_2S production but not in S-reducing bacteria in response to S supplementation. This supports our lack of abundance changes in S-reducing bacteria, suggesting that S-reducing bacteria may respond to high SO_4^{2-} through increased enzymatic activity rather than a significant shift in population abundance. Conversely, fiber utilizing rumen microbial species have been shown to react to SO_4^{2-} with notable shifts in abundance (Morrison et al., 1990).

A lack of congruency is evident across many S-related studies. There are many factors that influence dietary S requirements, such as diet composition, metabolic status, livestock species and breed type, as well as rumen microbial fermentation and protein synthesis. Additionally, microbial and host response to SO_4^{2-} has been shown to vary. While there were some differences in population abundance patterns, the reoccurrence of specific species across trials suggest that these microbes are particularly responsive to a high SO_4^{2-} environment. Furthermore, alterations in abundance or the adaptation of certain *Prevotella and Butyrivibrio* species may influence individual host animal ability to tolerate high SO_4^{2-} drinking water. Although no differences for S-reducing bacteria were apparent in the DNA sequencing data, the microbial differences that were observed could lead to a greater understanding of the relationship between the rumen microbiome and the host animal response to high levels of SO_4^{2-}.

IMPLICATIONS

Because rumen microbes are responsible for the degradation of consumed feedstuffs, ruminant livestock possess the unique ability to reduce dietary SO_4^{2-}. As a result, ruminant livestock are also subject to health complications associated with the overproduction of H_2S by the SO_4^{2-} reducing bacteria. Results from the current study demonstrate that rumen microbe abundances shift in response to high SO_4^{2-} water, and also indicate that certain microbial species may be particularly responsive to SO_4^{2-} and play an important role in host response. Because ruminant livestock in the western U.S. frequently encounter high SO_4^{2-} drinking water sources, it is imperative that feasible and effective treatment and management strategies be developed for affected livestock. A better understanding of the role of the rumen microbiome in host response to high dietary SO_4^{2-} is critical to this endeavor.

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Table 1. Abundance least-squares means[1] of taxa significant for sampling day effect in lambs administered high SO_4 water for a 28 d trial period (Trial 1).

	Sampling Day			
Taxa	d 0	d 7	d 28	*P*-values
Prevotella ruminicola	125.85[a]	166.96[b]	178.69[b]	<0.001
Prevotella genomospecies	8.75[a]	13.98[b]	13.52[b]	0.001
Prevotella albensis	3.05[a]	5.04[b]	5.66[b]	0.004
Prevotella nigrescens	1.51[ab]	2.33[a]	0.53[b]	0.004
Butyrivibrio fibrisolvens	2.08[a]	1.01[ab]	0.16[b]	0.001

[1]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.

[a,b]Least squares means without a common superscript differ ($P < 0.05$).

Table 2. Abundance least-squares means[1] of taxa significant for sampling day effect in lambs administered high SO_4 water for a 28 d trial period (Trial 2).

	Sampling Day			
Taxa	d 0	d 7	d 28	*P*-values
Prevotella ruminicola	141.69[a]	111.54[b]	110.38[b]	<0.001
Prevotella genomospecies	1.57[a]	0.55[ab]	0.21[b]	0.001
Prevotella genomospecies	6.41[a]	2.59[b]	3.51[b]	<0.001
Prevotella albensis	4.70[a]	7.30[ab]	8.89[b]	0.015
Prevotella nigrescens	0.33[a]	0.17[a]	0.99[a]	0.014
Butyrivibrio fibrisolvens	1.08[a]	1.17[a]	0.241[a]	0.020

[1]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.

[a,b]Least squares means without a common superscript differ ($P < 0.05$).

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Effects of grazing intensity and advancing season on chemical composition and in vitro organic matter disappearance in steers grazing mixed-grass prairie

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ABSTRACT: A study was conducted to evaluate the influence of advancing season and grazing intensity on dietary chemical composition and in vitro organic matter disappearance (IVOMD) in beef steers grazing mixed-grass prairie in the Missouri Coteau of south central North Dakota. Five sampling periods were conducted from mid-May to early September 2015. Twelve ruminal cannulated crossbred steers were used to collect diets while 188 crossbred steers were used to maintain specific grazing intensities on 12 pastures. Treatments were light (LT), moderate (MOD), heavy (HVY), and extreme (EXT) grazing intensities. Each treatment was assigned to 3 pastures. Grazing treatment × sampling period interactions were not present ($P \geq 0.29$) for all variables measured except IVOMD ($P < 0.01$). There were no main effects of grazing treatment for NDF, ADF, total N, soluble N (SN), insoluble N (IN), and ADIN. Responses to grazing season were evaluated with linear, quadratic, and cubic contrasts. Neutral detergent fiber increased linearly ($P < 0.01$) and cubically ($P = 0.01$), while ADF tended ($P = 0.17$) to increase linearly with advancing season. Dietary N decreased linearly ($P < 0.01$), quadratically ($P = 0.01$), and cubically ($P = 0.01$). Soluble N and IN expressed a linear ($P < 0.001$) and quadratic ($P = 0.03$) decrease across advancing season, while IN also showed a cubic response ($P < 0.001$). Acid detergent insoluble N did not change as season advanced ($P > 0.14$). In vitro OM digestibility decreased from May to September ($P < 0.01$) in all sampling periods, but did not show any trends across treatments ($P = 0.82$). However, IVOMD did show a treatment × period interaction ($P < 0.01$). In summary, these data indicate increases ($P < 0.001$) in dietary NDF and decreases ($P < 0.001$) in N, SN, IN, and IVOMD with advancing season. These data suggest seasonal factors are a more important driver of grazed masticate forage nutrient composition than the grazing intensities evaluated in this study.

Key words: dietary nutrient composition, grazing intensity, season

INTRODUCTION

Dietary chemical composition of grazed forage, when coupled with forage intake and digestion are important factors in rangeland based cattle production systems. We know that as forage maturity increases, dietary CP, digestibility, and intake often decline, while dietary fiber usually increases (Olson et al., 1994; Johnson et al., 1998; McCollum et al., 1985; Adams et al., 1987; Cline et al., 2009). Bryant et al. (1970) found that if grazing pressure is intense enough to cause a low availability of herbage, quality of herbage ingested deceases due to the reduced opportunity for selective grazing. Furthermore, as grazing intensity increases, diet quality decreases (Cook et al., 1953; Pieper et al., 1959).

It is common for beef cattle operations to maintain herds on native grass to reduce input costs of harvested and purchased feeds. Therefore, modulating cattle stocking rates on pasture is a common management tool used to achieve long-term goals of optimizing forage use, livestock production, and agroecosystem sustainability (Derner et al., 2008; Biondini et al., 1998; Hart et al., 1988). However, information regarding the impact of grazing intensity on forage intake and digestion by cattle grazing mixed-grass prairie is lacking. Hence, our objectives were to evaluate the effects of advancing season and grazing intensity on diet chemical composition and in vitro OM digestibility (**IVOMD**) by steers grazing mixed-grass prairie in the Missouri Coteau of south central North Dakota.

MATERIALS AND METHODS

Animals

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. Angus cross beef steers (n = 188; 320 ± 35.2 kg initial BW) were used to establish grazing pressure, and 12 ruminal cannulated steers (272 ± 33.6 kg) co-grazed with the non-cannulated animals. All steers had free access to water and trace mineral salt blocks (salt 95.5 to 98.5%, zinc 3,500 mg/kg, iron 2,000 mg/kg, manganese 1,800 mg/kg, copper 280 to 420 mg/kg, iodine 100 mg/kg, and cobalt 60 mg/kg; American Stockman Hi-Salt with EDDI; North American Salt Company, Overland Park, KS). Steers were fed dried distillers grains with solubles (**DDGS**) daily at sunrise at 0.3% of their BW. All animals were weighed every 28 d to determine gains as the grazing season progressed as well as used to adjust DDGS fed. All steers were implanted with Revalor-G (40 mg of trenbolone acetate and 8 mg estradiol; Intervet Inc., Millsboro, DE) 1 d before being turned out on pasture.

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Experimental Design and Treatments

The grazing trial was conducted at the Central Grasslands Research Extension Center (CGREC) located on the Missouri Coteau in south-central North Dakota. The study site had been divided into 12 pastures of approximately 12.9 ha each in 1989. Cattle grazed from May 15 to September 11 of 2015. Patton and Nyren (2014) reported the botanical composition of the plant communities at the study site the year before this study. The most common grasses in 2014 were Kentucky bluegrass (*Poa pratensis L.*), western wheatgrass (*Pascopyrum smithii A.*), sun sedge (*Carex inops*), green needlegrass (*Nassella viridula*), obtuse sedge (*Carex obtusata Lilj.*), and blue grama (*Bouteloua gracilis).* Common forbs were heath aster (*Symphyotrichum ericoides*), common dandelion (*Taraxacum officinale*), and western yarrow (*Achillea millefolium L.)*. Buckbrush (*Symphoricarpos occidentalis*) was the only common shrub. Steers were stocked at densities so that at the end of the grazing season 65 (**LT**), 50 (**MOD**), 35 (**HVY**), and 20% (**EXT**) of an average annual above ground biomass remained at the end of the grazing season. Each of the cannulated steers was assigned to a pasture at random, each treatment having 3 pastures. Animals were removed at the end of the grazing season when forage utilization on half of the pastures had reached desired grazing intensity.

Five, 10 d collection periods were conducted for May, June, July, August, and September. Sampling periods began with collection of diet samples. At sunrise, cannulated steers were restrained and subjected to total ruminal evacuation. Ruminal digesta was physically removed from each cannulated steer and the rumen was then double rinsed with water to assure complete removal of contents. Steers were then allowed to graze on their assigned pastures for 30 to 45 min. Then ruminal masticate samples were removed, labeled, and immediately placed on ice. Previously collected ruminal contents were placed back in the animal. All samples were then frozen at -20˚C for later analysis.

Masticate samples were lyophilized (Genesis 25LL, Virtis, Gardiner, NY). Dry matter, ash, and CP were determined using AOAC (1990). Neutral detergent fiber and ADF of diet samples were determined using ANKOM procedures (ANKOM, Macedon, NY). Acid detergent insoluble N was calculated as N remaining in the ADF residue. Soluble N was extracted with 0.15 M NaCl according to the procedure of Waldo and Goering (1979). In vitro OM digestibilities (Tilley and Terry, 1963) were conducted to determine IVOMD. Masticate forage and ruminal fluid collected from each animal was used for in vitro determinations.

Statistical Analysis

Chemical composition and IVOMD were analyzed as a repeated measures design using a mixed model approach in SAS (SAS Inst. Inc., Cary, NY). Effects for sampling period, grazing treatment, and period × treatment interactions were included in the model. In the absence of interactions, orthogonal contrasts were used to determine linear, quadratic, and cubic, responses across the grazing season (sampling period). Sampling period × grazing treatment interactions ($P \leq 0.05$) were detected for IVOMD; therefore, the simple effect means were separated using the LSMEANS statement in SAS. The procedures of SAS were used for all statistical analysis and *P*-values ≤ 0.05 were considered different.

RESULTS AND DISCUSSION

Diet Analyses

No treatment × period interactions ($P > 0.05$) were observed, with the exception of interactions of IVOMD which will be discussed later in the Results and Discussion. Therefore, main effect means are reported for grazing intensity treatment and grazing period (Table 1). Organic matter, NDF, and ADF of cattle diets were not affected ($P > 0.05$) by grazing intensity. Crude protein, total N, soluble N (**SN**), insoluble N (**IN**), and ADIN also did not differ between grazing intensity treatments ($P > 0.05$).

Table 1 shows the effects of grazing intensity and advancing season on chemical composition of diet as well as IVOMD. Neutral detergent fiber and ADF changed with advancing season ($P < 0.01$). These results coincided with Olson et al. (1994) for south central North Dakota, Johnson et al. (1998) for western North Dakota, and McCollum et al. (1985) for south central New Mexico. Neutral detergent fiber increased with advancing season ($P < 0.01$ and $P = 0.01$, respectively for a linear and cubic response) and ADF tended ($P = 0.17$; linear) to increase as season advanced. These responses are supported by previous data from south central North Dakota (Olson et al., 1994), western North Dakota (Johnson et al., 1998), as well as south central New Mexico (McCollum et al., 1985) who observed similar responses.

Nitrogen (% of OM) decreased linearly ($P < 0.01$), quadratically ($P < 0.01$), and cubically ($P < 0.01$) as season advanced. Typically, forage masticate N concentration declines with increasing forage maturity associated with advancing season. Such was the case in our study and the work of others within the region (Olson, et al., 1994; Johnson, et al., 1998; Cline et al., 2009). Soluble N decreased ($P < 0.01$) in a linear fashion, whereas, IN data were represented by declining linear, quadratic, and cubic responses ($P < 0.01$). McCollum et al. (1985) also found that N, SN, and IN decreased with advancing grazing season. In the present study, ADIN was not impacted by advancing season. However, Cline et al. (2009) observed an increase in ADIN from late June to mid-November.

IVOMD

There was a sampling period × by grazing intensity interaction ($P = 0.01$; Table 1); therefore, interactive means are discussed (data not shown). In vitro OM digestibility decreased from May to September ($P < 0.05$) in all grazing intensities. In May, IVOMD was similar across all grazing intensities ($P > 0.05$). In June, LT and MOD had greater ($P < 0.05$) IVOMD compared with HVY, while EXT was

similar to all grazing intensities (75.7, 73.4, 62.9, and 69.1 ± 2.8 %, respectively). In July, LT had the lowest and HVY the greatest IVOMD ($P < 0.05$). In August, LT had similar ($P > 0.05$) IVOMD compared with all other grazing intensities, while MOD was lower ($P < 0.05$) than HVY. In September, IVOMD was 52.5, 44.9, 50.3, and 42.8 ± 5.1 %; $P > 0.05$) for LT, MOD, HVY, and EXT grazing intensities.

IMPLICATIONS

The results of this study demonstrate that grazed forage by beef cattle in the Missouri Coteau increase in fiber and decrease in N as season advances. Grazing intensity had little impact on grazed forage nutrient composition. Consequently, previously observed differences in livestock production due to grazing intensity in the Missouri Coteau must be driven by changes in dietary intake or in vivo digestion. Additional research accessing changes in intake and rates of digestion are needed.

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Table 1. Effects of grazing intensity and advancing season on dietary chemical composition, and in vitro OM digestibility (IVOMD) in steers grazing mixed-grass prairie

	Grazing Intensity (TRT)[1]					Grazing Period (PD)[2]						*P*-value[3]					
Item	LT	MOD	HVY	EXT	SEM[4]	1	2	3	4	5	SEM[5]	TRT	PD	TRT × PD	L	Q	C
No. of Observations	15	15	15	15	-	12	12	12	12	12	-	-	-	-	-	-	-
OM, %	81.3	82.2	80.6	82.3	1.49	74.6	82.4	83.9	83.7	83.2	3.08	0.83	0.05	0.44	0.02	0.02	0.16
	----------Percentage of OM----------																
NDF	67.5	69.6	70.7	65.6	2.05	58.4	69.9	67.5	70.7	75.2	3.81	0.34	<0.01	0.89	<0.01	0.32	0.01
ADF	38.5	40.2	41.5	36.1	1.85	37.2	38.5	37.1	39.5	43.1	4.05	0.25	<0.01	0.87	0.17	0.33	0.44
CP	18.7	17.7	17.8	19.6	0.63	29.9	18.3	16.9	14.9	12.3	0.86	0.18	<0.01	0.63	<0.01	<0.01	<0.01
N	2.99	2.84	2.85	3.14	0.10	4.78	2.92	2.70	2.38	1.97	0.14	0.18	<0.01	0.63	<0.01	<0.01	<0.01
Soluble N	0.70	0.73	0.69	0.82	0.06	1.08	0.81	0.66	0.57	0.55	0.09	0.36	<0.01	0.29	<0.01	0.03	0.73
Insoluble N	2.28	2.11	2.16	2.32	0.08	3.70	2.11	2.05	1.81	1.42	0.12	0.22	<0.01	0.59	<0.01	<0.01	<0.01
ADIN	0.48	0.42	0.44	0.47	0.04	0.52	0.37	0.47	0.44	0.45	0.06	0.77	0.26	0.93	0.70	0.31	0.15
IVOMD	60.4	62.6	63.4	60.6	2.62	75.9	70.3	62.1	52.8	47.6	3.35	0.82	<0.01	0.01	0.97	0.97	0.14

[1]LT = light, MOD = moderate, HVY = heavy, and EXT = extreme grazing intensities.

[2]Grazing period collections were 1 (May 11 to 22), 2 (June 10 to 19), 3 (July 8 to 17), 4 (August 5 to 14), and 5 (September 2 to 11).

[3]Significance level of the F-test for treatment (TRT), period (PD), treatment by period (TRT × PD), linear (L), quadratic (Q), and cubic (C) effects for items.

[4]Standard error of mean for grazing intensity, n = 15. Most conservative standard error mean values were used.

[5]Standard error of mean for grazing period, n = 12. Most conservative standard error mean values were used.

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Altering the time of vaccination against respiratory pathogens to enhance vaccine efficacy, health, and performance of feedlot cattle

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ABSTRACT: Ninety Angus × Hereford calves were ranked by gender, BW, and age, and assigned to 1 of 3 vaccination schemes against respiratory pathogens: 1) vaccination at weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) vaccination 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) vaccination 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30). From d -15 to 6, calves were maintained as a single group on pasture. On d 7, calves were placed according to treatments into 1 of 18 drylot pens (6 pens/treatment; 5 calves/pen) for a 30-d preconditioning period, and fed a forage-based diet. On d 30, calves were transported 1,440 km in a livestock trailer and returned to different drylot pens for a 45-d receiving period. Calves were fed a forage + concentrate diet during the receiving period. Blood samples and BW were collected on d -15, 0, 15, 30, 45, 60, and 75. Additional BW was collected on the day after blood sampling so 2 consecutive BW were recorded and averaged. There were no treatment effects on BW pre-weaning, at weaning, or during the preconditioning and receiving periods ($P \geq 0.59$). The EARLY calves had less ($P \leq 0.05$) ADG pre-weaning, however had greater ($P \leq 0.04$) ADG during feedlot receiving compared to the other treatments. During preconditioning, CON had greater ($P = 0.05$) DMI compared with EARLY and DELAYED calves, but there were treatment effects ($P \geq 0.20$) on forage or concentrate DMI during the feedlot receiving. There were no treatment effects ($P \geq 0.16$) on G:F, morbidity, or mortality. By 15-d after initial vaccination, DELAYED calves had the greatest ($P < 0.01$) antibody titers against *Mannheimia haemolytica*, and EARLY calves had the lowest ($P \leq 0.05$) antibody titer against this pathogen. By revaccination, there was no difference ($P = 0.82$) between DELAYED or CON for antibody titers against *M. haemolytica* titers, while EARLY titers remained lower ($P < 0.01$) compared with both treatments. However by 45-d after initial vaccination, EARLY calves had the greatest antibody titers ($P \leq 0.05$) against *M. haemolytica*, which remained the greatest until 60-d after initial vaccination. These data suggest that while pre-weaning ADG may be inhibited by vaccination before weaning, vaccination before weaning and revaccination before feedlot receiving can improve overall antibody titer to *M. haemolytica* and ADG during feedlot receiving.

Keywords: feeder cattle, health, performance, vaccination.

INTRODUCTION

Bovine respiratory disease (**BRD**) is the most common and costly disease of feedlot cattle in the US (NASS, 2006), and costs the US cattle industry approximately $ 1 billion annually (Griffin, 1997). These economical losses include cattle morbidity, wasted feed resources, purchase of pharmaceuticals, and reduced performance of morbid cattle (Loerch and Fluharty, 1999). Preconditioning programs that include vaccination for viral and bacterial agents that cause BRD are one of the most effective management methods that mitigate the incidence of this disease in feeder cattle (Martin et al., 1999; Duff and Galyean, 2007).

In typical western US preconditioning programs, calves receive vaccination against BRD pathogens at weaning and are revaccinated 30 days later at feedlot entry (England et al. 2009; Bohnert and Johnson, 2010). However, weaning and feedlot entry are two of the most stressful situations encountered by feeder cattle (Araujo et al., 2010) and vaccine efficacy can be reduced if administered to highly-stressed animals (Blecha et al., 1984; Loerch and Fluharty, 1999). Excessive stress leads to immunosuppression (Duff and Galyean, 2007) and impairs the vaccine-induced effects that infer protective immunity to cattle (Callan, 2001). In addition, vaccination against BRD pathogens elicits innate immune responses known to impair cattle performance, such as inflammatory and acute-phase reactions (Tizard, 2004; Johnson, 1997). For example, vaccination against *Mannheimia haemolytica* has been shown to reduce average daily gain and feed efficiency in feedlot steers during the 2 weeks subsequent to vaccination (Arthington et al., 2013). Hence, altering the time of vaccination/revaccination against BRD is a strategy that has been only partially investigated (Richeson et al., 2008).

The objective of this experiment was to compare the effects of anticipated, delayed, or vaccination at the time of weaning and feedlot entry on vaccine efficacy, health, and performance variables of feeder cattle.

MATERIALS AND METHODS

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns station). All animals 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 treatments. Ninety Angus × Hereford calves were utilized for this experiment. All calves 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 45 d of age. On d -18, calves were ranked by gender (steers, n = 69; heifers, n = 21), dam's parity, BW, and age (BW = 215 ± 4 kg, age = 184 ± 18 d), and assigned to 1 of 3 treatments: 1) vaccination at weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) vaccination 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) vaccination 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30). Vaccines administered were against *Clostridium* (One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus (TSV-2; Zoetis), infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (Bovi-Shield Gold One Shot; Zoetis).

From d -15 to 6, calves were maintained as a single group on pasture. On d 7, calves were placed into 1 of 18 drylot pens (6 pens per treatment) with 5 calves per pen for a 30-d preconditioning period and fed alfalfa-triticale hay ad libitum. On d 30, calves were transported 1,440 km in a livestock trailer and returned to different drylot pens for a 45-d receiving period. Calves were fed alfalfa-triticale hay and concentrate supplement twice a day to mimic a total mixed ration. During feedlot receiving, concentrate was originally fed at 2.7 kg/steer daily (DM basis) and gradually increased to 9.5 kg/steer daily (DM basis).

Sampling. Blood samples were collected on d -15, 0, 15, 30, 45, 60, and 75 via jugular venipuncture in heparinized blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA) in order to ensure that calves from each treatment group were sampled at the time of vaccine administration, as well as 15 and 30 later to estimate vaccine efficacy via serum antibody titers or concentrations (Callan, 2001). Tubes were then placed on ice and centrifuged at 2,400 × g for 30 min at 4°C temperature for plasma collection. Plasma was stored in duplicate at −80°C, and analyzed for *Mannheimia haemolytica* titers.

Calf BW was recorded d -15, 0, 15, 30, 45, 60, and 75, in addition to the day after blood sampling, so 2 consecutive BW were recorded and averaged. Forage and concentrate orts were collected daily from d 7 to d 75 by pen, and analyzed for DMI to calculate DMI and G:F.

Statistical analysis. For BW, ADG, health parameters, and titer variables, data were analyzed with calf as the experimental unit, and calf(pen × treatment × gender) as random variable. Pen was the experimental unit for DMI and G:F variables with pen(treatment) as the random variable. All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.). The model statement contained the effects of treatment, day, gender, and all resultant interactions. The specified term in the random statement was day, and the subject was pen(treatment) for DMI and calf(pen × treatment × gender) for the remaining variables. Significance was concluded if $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10.

RESULTS AND DISCUSSION

Body weight and ADG parameters can be found in Table 1. There was no difference ($P \geq 0.76$) in initial pre-weaning BW on d -15 or weaning weight on d 0. However, EARLY calves had decreased ($P \leq 0.05$) ADG during the pre-weaning period compared to CON or DELAYED calves. This supports research by Arthington et al. (2013) who observed suppressed ADG for 2 weeks in vaccinated heifers. Final preconditioning BW and preconditioning ADG were similar ($P \geq 0.65$) among treatments. After transportation, there was no difference ($P \geq 0.59$) in BW upon entrance to the feedlot or at the end of the receiving period. However, EARLY calves had the greatest ($P \leq 0.05$) ADG during the receiving period. This is contradictory to results seen by Richeson et al. (2008), who observed greater BW gain in calves that received a delayed vaccination 14-d after entrance the feedlot vs. calves that received a vaccine upon feedlot arrival.

Feed intake and efficiency data can be found in Table 2. A treatment effect was detected ($P = 0.05$) for forage DMI during the preconditioning period, as DMI was greatest ($P \leq 0.05$) for CON calves with no difference ($P > 0.05$) between EARLY or DELAYED. Additionally, a treatment × day interaction was detected ($P \leq 0.01$) as forage consumption decreased ($P \leq 0.001$) for EARLY and DELAYED calves from d 15 to 18. This decrease in DMI was to be expected, as EARLY and DELAYED calves received a vaccination at this time. Marques et al., (2014) reported a similar reduction in DMI for 2 d post-vaccination to *Mannheimia haemolytica.* No differences were detected ($P > 0.05$) in forage, concentrate, or total DMI during the feedlot receiving period, however there was a tendency ($P = 0.09$) for EARLY calves to consume more hay than CON calves. This is supported by Rodrigues et al. (2015) who observed a decrease in forage DMI after vaccination, while concentrate DMI was not affected. There were no treatment effects detected ($P \geq 0.16$) for G:F during either preconditioning or feedlot receiving.

Health parameter data can be found in Table 3. There were no treatment effects detected ($P > 0.05$) for morbidity or mortality data during either preconditioning or feedlot receiving. This may be due to the fact that morbidity rate during the receiving period was rather low compared to previous studies (Boyles et al., 2007; Richeson et al., 2008). This low morbidity rate is likely due to the fact that while calves were transported, they were brought back to the same facility with the same pen members, and were not exposed to calves from other sources in a new environment.

Vaccination titers to *Mannheimia haemolytica* are presented in Table 4 and are relative to the day of the first vaccination (d 0). There were no treatment effects detected on d 0, however by 15-d post-vaccination, DELAYED calves had the greatest ($P < 0.001$) antibody titers and EARLY calves had the lowest ($P \leq 0.05$). By the time of the booster vaccination 30-d after the initial vaccine, there was no difference between CON and DELAYED ($P > 0.05$), and EARLY titers remained the lowest ($P < 0.01$). However, by d 45, EARLY calves had the greatest ($P \leq 0.05$) titers and DELAYED had the lowest ($P \leq 0.05$). This increase in EARLY titers was maintained 60-d after initial

vaccination, as they were greater ($P < 0.01$) than either CON or DELAYED titers. As calves were still with their dams and nursing when EARLY calves received their initial vaccine, the slow response observed may be explained by remaining maternal antibodies. Maternal antibodies begin to decline rapidly when the calf is about 4-5 months of age, however maternal antibodies can interfere with the response to vaccination (Funnell, 2011). Downey et al. (2013) reported an increase in response to vaccination when calves were weaned at an initial vaccine as opposed to the booster, as maternal antibodies negatively affected immune response. Therefore, it may be possible that the results seen in the present study inhibited the EARLY treatment group from exhibiting a strong immune response, but were able to 30-d later at the booster vaccine.

IMPLICATIONS

Administering vaccines against respiratory pathogens 15-d pre-weaning and 15-d pre-feedlot entry did not inhibit weaning weight or feedlot performance, and increased ADG during feedlot receiving. Additionally, early vaccination provided greater final antibody titer to *Mannheimia haemolytica*. Delaying vaccination until 15-d after weaning and 15-d after transportation provided similar performance and titers as current industry protocol. Based on these results, vaccinating calves before weaning and feedlot entry appear to be a viable option to increase calf performance and overall immune response to vaccination during feedlot receiving.

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Table 1. Performance parameters of calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30).[1]

Item	EARLY	CON	DELAYED	SEM	*P-value*
BW, kg					
Pre-weaning (d -15)	215	215	215	4	0.99
Weaning (d 0)	220	225	223	4	0.76
Final preconditioning (d 29)	228	234	231	4	0.65
Feedlot entry (d 30)	210	215	215	4	0.59
Final feedlot receiving (d 75)	275	273	270	4	0.78
ADG, kg/d					
Pre-weaning (d -15 to 0)	0.38[a]	0.65[b]	0.55[b]	0.08	0.04
Preconditioning (d 1 to 30)	0.26	0.30	0.26	0.07	0.88
Feedlot receiving (d 31 to 75)	1.46[a]	1.29[b]	1.24[b]	0.06	0.04

[1] Means with different superscripts differ ($P \leq 0.05$).

Table 2. Feed intake (DM basis) of calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30).[1]

Item	EARLY	CON	DELAYED	SEM	*P-value*
DMI, kg/d					
Preconditioning (d 7 to 30)					
Hay	5.08[a]	5.60[b]	5.03[a]	0.16	0.05
Feedlot receiving (d 31 to 75)					
Hay	4.10	3.67	3.98	0.16	0.20
Concentrate	3.72	3.71	3.71	0.07	0.99
Total	7.81	7.39	7.69	0.18	0.28
G:F (g/kg)					
Preconditioning (d 7 to 30)	92.0	92.7	84.9	12.9	0.89
Feedlot receiving (d 31 to 75)	198.5	193.4	175.0	8.6	0.16

[1] Means with different superscripts differ ($P \leq 0.05$).

Table 3. Health responses of calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30).

Item	EARLY	CON	DELAYED	SEM	*P-value*
Morbidity, %					
Preconditioning, % (d 0 to 30)	27.9	34.5	32.3	9.5	0.88
Feedlot receiving, % (d 31 to 75)	2.1	16.5	9.3	5.3	0.17
Mortality, %	0.0	4.4	4.4	4.5	0.73
Preconditioning, % (d 0 to 30)	-	-	-	-	-
Feedlot receiving, % (d 31 to 75)	0.0	4.4	4.4	4.5	0.73

Table 4. *Mannheimia haemolytica* titers in calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30).[1] feeder calves in relation to day of first vaccination. Values are reported relative to day of first vaccination. [1]

Item	**EARLY**	**CON**	**DELAYED**	**SEM**	***P-value***
Titer					
d 0 (first vaccination)	0.03	0.03	0.11	0.07	0.60
d 15	0.18[a]	0.38[b]	0.71[c]	0.07	<0.01
d 30 (second vaccination)	0.21[a]	0.51[b]	0.49[b]	0.07	<0.01
d 45	0.80[a]	0.59[b]	0.43[c]	0.07	<0.01
d 60	0.55[a]	0.32[b]	0.25[b]	0.07	<0.01

[1] Means with different superscripts differ ($P \leq 0.05$).

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Evaluation of genetic structure across five U.S. climate zones using prominent AI sires of two British *Bos taurus* breeds

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ABSTRACT: Cattle performance in diverse climates can be problematic if they cannot adapt to climate variability. Previous research showed Hereford cattle to have genetic substructure associated with U.S. climates: Cool Arid (CA), Cool Humid (CH), Transition Zone (TZ), Warm Arid (WA), and Warm Humid (WH). Allele frequencies of 66 SNP from BovineSNP50 (Illumina BeadChip) were associated with the following traits: mature cow body weight, heat stress, milk yield, heifer conception rate, and early embryonic survival. Knowledge of these genotype to phenotype associations were queried from CattleQTLdb. The GENALEX (6.501) software was used to estimate population genetic results. To characterize the diversity in another British *Bos taurus* breed, population genetic characteristics were estimated in Red Angus bulls (n = 175) that were included in the 2000 Bull Project. Similar software, climate zone regions, and SNP were used in the analyses. The number of sires in the climates zones of CA, CH, TZ, WA, and WH were 126, 32, 11, 5 and 1, respectively. We hypothesized Red Angus bulls would possess genetic substructure across the five climatic zones as observed in Hereford bulls. ARLEQUIN (3.5.2.2) software was used to estimate Hardy-Weinberg Equilibrium (HWE) and conduct an analysis of molecular variance for genotype to phenotype associations. The number of significant ($P < 0.05$) SNP for the traits of milk yield, early embryonic survival, and mature cow body weight were 4, 1, and 1, respectively. Based on the results and genotypes from the bulls studied in the 2000 Bull Project, we reject our hypothesis that Red Angus bulls possess genetic substructure similar to Hereford bulls across five U.S. climate zones. These results provide evidence to suggest that Red Angus cattle in the U.S appear to be preferred in beef production systems in cooler climate zones; whereas Hereford cattle populate these regions as well as drier climate zones.

Key words: *Bos taurus*, genetic diversity management, molecular markers

INTRODUCTION

Global climatic change makes understanding genotype by environment interactions (G x E) important for sustaining livestock production. Manel and Holderegger (2013) reported that animal population decline is directly linked to the loss of intra-species genetic diversity. Furthermore, Boettcher et al. (2015) stated diverse animal genetic resources allows for more opportunities to adapt to severe climates. When livestock experience warming climatic events, heat stress occurs and adverse effects on performance are possible. These decreases in performance are typically observed in traits such as milk yield, reproduction, and feed intake (Holter et al., 1997; West, 2003; and Bohmanova et al., 2007).

Krehbiel et al. (2015) reported that Hereford cattle appeared to have genetic substructure needed to perform across the five major climate zones of the U.S. This genetic substructure was determined with allele frequencies of 66 SNP associated with traits that could be influenced by climate zone (i.e., milk yield, mature cow body weight, heat stress, early embryonic survival, etc.). This substructure means that there were allele frequencies of the SNP that were unique to each of the climates zones. To characterize the diversity in another British *Bos taurus* breed, population genetic parameters were estimated in Red Angus cattle. We hypothesized Red Angus bulls would possess genetic substructure across the five U.S. climate zones similar to Hereford. Improving our knowledge base for this breed will assist in breeding decisions.

MATERIALS AND METHODS

Cattle studied in this project were primarily from the 2000 Bull Project (Kuehn, et al., 2011), which involved the most influential AI sires within the U.S. Beef Industry. Two-hundred and eighteen of the 278 Hereford sires were also included in the 2000 Bull Project. The bias created by Line 1 Herefords was minimized via pedigree analysis. The EPD accuracy for eight growth and carcass traits of the Hereford and Red Angus bulls in this study were 54.48 ± 4.49 and 79.91 ± 5.29 (RAAA, 2016; AHA 2016), respectively. Bulls were genotyped using the BovineSNP50 Bead Chip (54,001 SNP; Matukumalli et al., 2009). Red Angus bulls (n = 175) were assigned to one of the five climate zones in the U.S. based upon temperature, humidity, and breeder location. The climate zones were

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Cool Arid (CA), Cool Humid (CH), Transition Zone (TZ), Warm Arid (WA), and Warm Humid (WH). These were the same zones that were assigned to Hereford bulls in Krehbiel et al. (2015).

From the BovineSNP50 genotype data, a subset of 100 SNP were selected based on their association with the following traits: mature cow body weight, heat stress, heifer conception rate, milk yield, and embryonic survival. These traits were queried from CattleQTLdb (Hu et al., 2013) based on their logical likelihood of being responsive to climate change. The subset of 100 SNP was reduced to 66 SNP due to duplication across traits, or lack of information of the SNP. The GENALEX 6.501 software (Peakall and Smouse, 2012) was used to estimate the allele frequencies of the 66 SNP. Due to limited data in the WH region (n = 1), this region was omitted from the analysis. ARLEQUIN 3.5.2.2 software (Excoffier and Lischer, 2010) was used to test Hardy-Weinberg Equilibrium (HWE) and (or) loci detected to be under selection (DLS). We performed a HWE locus-by-locus test with 1,000,000 Markov chain steps and 10,000 dememorisation steps. A DLS analysis was performed with 100 simulated demes and 50,000 coalescent simulations. The SNP considered significant ($P < 0.05$) in the HWE and DLS analyses were further evaluated for genetic differentiation through an analysis of molecular variance (AMOVA) with 10,000 permutations for significance and 1000 permutations for Mantel test. In addition, a population pairwise matrix based on Wright's Fixation Index (F_{st}) that estimates loss in genotype heterozygosity was constructed.

RESULTS

Study of prominent Red Angus AI sires revealed 22 SNP deviated significantly from HWE or DLS analyses across the climatic zones. These results were used to construct a population pairwise matrix and suggested genetic similarities between the TZ and CA and WA climate zones (Table 1). The number of significant ($P < 0.05$; AMOVA) SNP for the traits of milk yield, early embryonic survival, and mature cow body weight were 4, 1 and 1, respectively (Fig. 1). Based on these six SNP, the allele frequencies of the WA zone was dissimilar from the other climate zones for mature cow body weight and early embryonic survival. Allele frequencies of the CH zone were intermediate for most milk yield SNP.

Two SNP were found to be significantly ($P < 0.05$; AMOVA) divergent for both Red Angus and Hereford bulls. These SNP were known to be associated with the traits of mature cow body weight and milk yield. The contrasting allele frequencies of these two SNP are presented in Fig. 2.

DISCUSSION

Allele frequencies of SNP known to be associated with production traits likely to be influenced by climate change differed among the Red Angus and Hereford bulls from the 2000 Bull Project across five U.S. climate zones in the current study and the report of Krehbiel et al. (2015). The SNP previously associated with milk yield appeared to be most influenced by climate zone in Red Angus bulls, whereas SNP associated with milk yield and heat stress traits were most influenced by climate zones in Hereford bulls. Population genetic substructure was apparent based on allele frequencies and climate zones in Hereford bulls (Krehbiel et al., 2015). This substructure was almost non-existent in Red Angus bulls across climate zones; however, population pairwise comparisons of climate zones in both breeds revealed significant relationships between TZ and the other climate zones. This observation suggests that bulls used in TZ may possess robust genotypes that allow for adaptability across the other climate zones. As climates continue to change, hardy animals that balance production and fitness-related traits are needed to sustain production (Knap, 2005).

Allele frequency difference observed in Red Angus and Hereford bulls across climate zones may be indicative of diverse industry uses of these breeds. For example, heat stress SNP were not in HWE for the Hereford population among climate zones (Krehbiel et al., 2015). This result suggests potential natural or indirect artificial selection of Hereford bulls in warmer environments. Therefore, Hereford bulls may be more heat tolerant than Red Angus. Differences in allele frequencies of SNP associated with mature cow body weight and milk yield in both breeds may be indicative of the bulls utilized in the five U.S. climate zones. Although the 66 SNP included in this study were associated with traits influenced by climate to determine environment-driven diversity, it would be advantageous to determine the genetic diversity of Red Angus cattle by estimating allele frequencies of a whole-genome SNP panel.

Furthermore, the comparison of Red Angus and Hereford bulls in climate zones may give insight into the types of sires used in AI programs. The unique genetic substructure within the Hereford bulls (e.g. WH and WA regions) could be due to mating cows with natural service sires in extensive beef production system versus AI sires used in intensive production systems. Red Angus bulls used in the 2000 Bulls Project appeared concentrated to the northwestern region of the U.S. Therefore, distinct genetic substructure between the geographic regions, which may be due to their extensive use of AI, was difficult to detect.

Lastly, the American Hereford Association was established 73 years prior to the Red Angus Association of America in the U.S. (RAAA, 2016; AHA 2016). This may provide explanation as to why allele frequencies of the 66 SNP differed between the two breeds, and the reason the Red Angus breed most populates the northwestern region of the U.S. (i.e. the location of where the breed originated).

Genetic diversity to cope with climate change will become more important as breeds and species become more uniform. Napel et al. (2009) suggested multi-trait selection, heterosis, and natural selection all provide ways in which livestock can become more robust and less prone to environmental insults. Based on the results and genotypes from the bulls studied in the 2000 Bull Project, we reject our hypothesis that Red Angus bulls possess genetic substructure across five U.S. climate zones similar to Hereford bulls. These results may give insight to how the breeds have been utilized since their establishment in the

U.S., where Red Angus cattle appear to be greater represented in cooler climates and Hereford cattle appear to be distributed throughout the five U.S. climates zones.

IMPLICATIONS

Red Angus sires used in the 2000 Bull Project were concentrated in cool climates. This may be a result of the origin of Red Angus cattle in the northwestern region of the U.S. Robust AI programs used in these regions of the U.S. may also influence the population of Red Angus in the cooler climate regions. Nevertheless, studying and defining genetic diversity presents selection opportunities to combat weather, disease, and changing industry demands.

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Table 1. Average population differentiation (F_{st}) due to genetic substructure (22 SNP) in prominent Red Angus AI sires (n = 178) across climate zone in the U.S.

	Region			
Region	Cool Arid	Cool Humid	Transition Zone	Warm Arid
Cool Arid	0.00000			
Cool Humid	0.03656*	0.00000		
Transition Zone	-0.00620	0.02969*	0.00000	
Warm Arid	0.07816*	0.11105*	0.04566	0.00000

*$P < 0.05$

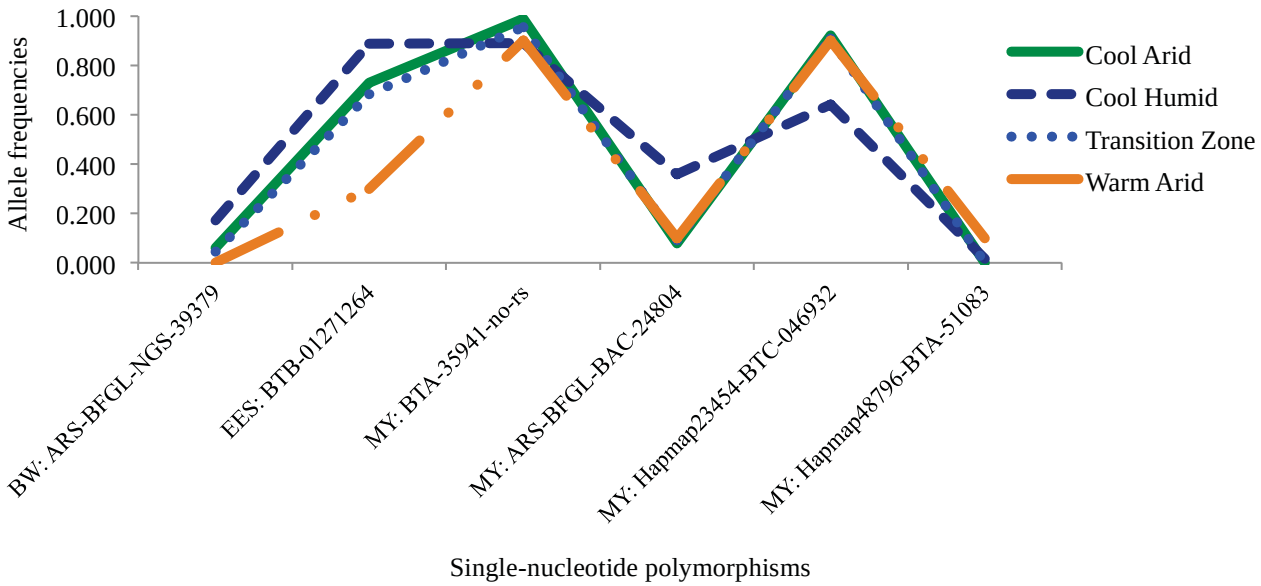

Figure 1. SNP allele frequencies of the A allele in Red Angus cattle for body weight (BW), early embryonic survival (EES), and milk yield (MY). These SNP were different ($P < 0.05$; AMOVA) among climate zones. The X-axis represents the trait followed by SNP name on BovineSNP50 manifest.

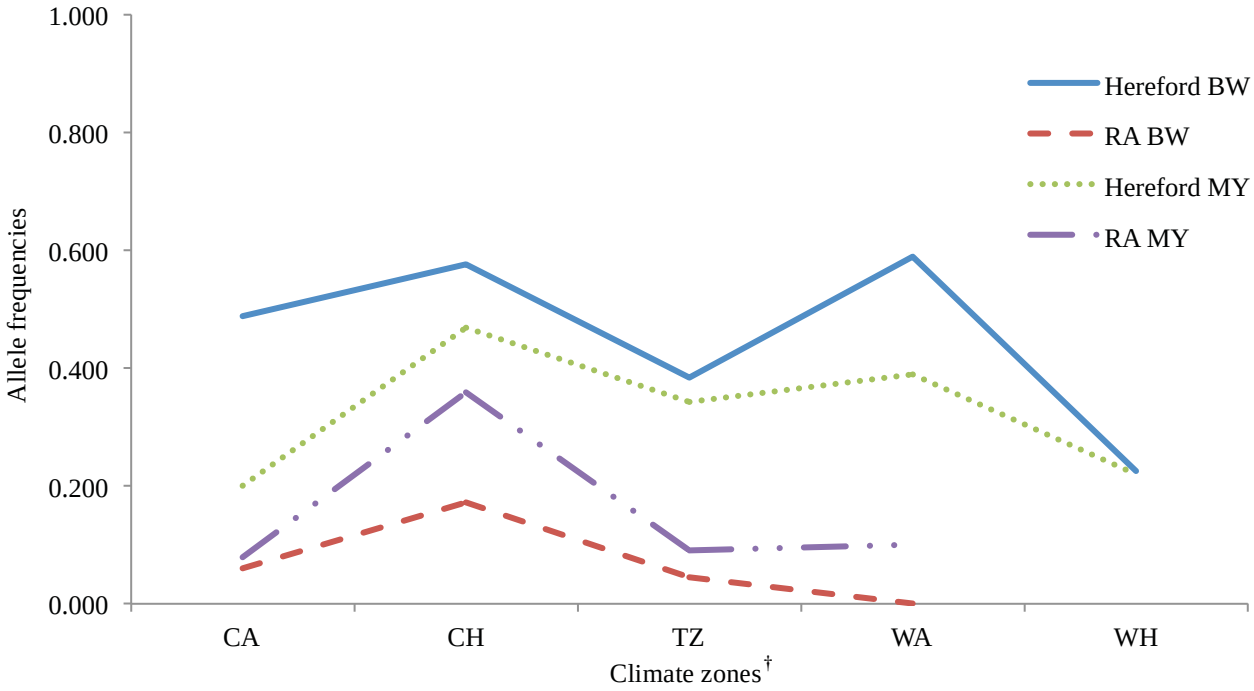

Figure 2. Allele frequencies of SNP known to be associated with body weight (BW) and milk yield (MY) that were significant ($P < 0.05$; AMOVA) across climate zones in prominent Red Angus (RA) and Hereford AI sires.

† CA = Cool Arid; CH = Cool Humid; TZ= Transition Zone; WA = Warm Arid; and WH = Warm Humid

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Effect of processing of supplemental corn on metabolizable protein of beef cows grazing winter wheat pasture

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ABSTRACT: Eight ruminally and duodenally cannulated, Angus-crossbred cows (587 + 49.0 kg) grazing winter wheat pasture (**WWP**) were used in a completely randomized design with the objective of evaluating effects of processing of supplemental corn (ground vs. steam-flaked) on forage intake and metabolizable protein. The experiment was conducted from March 23 through April 6, 2015. Cows grazed a single WWP with ground corn (**GC**) or steam-flaked corn (**SFC)** offered individually at 0.4% of BW, once daily at 0700 h. Forage DM intake and total DM intake were greater (P = 0.01) for SFC than for GC supplementation. Forage OM, CP, and NDF intake was greater (P = 0.01) for SFC than for GC supplementation. Total OM, CP, and NDF intake was greater ($P \leq 0.02$) for SFC than for GC supplementation. Although feed CP flow to the small intestine was not affected (P = 0.97) by corn processing method, microbial CP synthesis was greater (P = 0.01) for SFC than for GC supplementation. Therefore, total CP flow to the small intestine (metabolizable protein) was greater (P = 0.03) for SFC than for GC supplementation. Total tract digestibility of OM, CP, and NDF (expressed as g/d) were greater ($P \leq 0.02$) for SFC than for GC supplementation. In conclusion, forage intake, microbial protein synthesis, and metabolizable protein improved by steam-flaking as compared to grinding supplemental corn for cattle grazing WWP. Steam flaking as compared to grinding supplemental corn may improve performance of cattle grazing WWP by improving forage intake and microbial CP synthesis.
Key words: grain processing, metabolizable protein, steam flaked corn, winter wheat pasture

INTRODUCTION

Grazing winter wheat (*Triticum hybernum*) in the southern Great Plains of the United States has become a common practice and has great agricultural and economical importance as a dual purpose crop (Epplin et al., 2001). Winter wheat pasture (**WWP**) is a high-quality forage that contains over 70% digestible DM (Mader and Horn, 1986; Torell et al., 1999), and 25 to 30% CP (Johnson et al., 1973; Reuter and Horn, 2000). Dry matter and NDF concentrations tend to increase along with wheat spring growth (Branine and Galyean, 1990; Johnson et al., 1973). Moreover, more than 59% of N in wheat pasture is highly soluble (Vogel et al., 1989).

Microbial protein synthesis is principally affected by ruminal concentration of N-containing compounds and the quantity of OM available for fermentation (Hespell, 1979). Although WWP is high in N-containing compounds and readily digestible carbohydrate content, it is considered insufficient in energy due to the low structural carbohydrate content (Shroyer et al., 1993). Moderate amounts of grain supplementation have been successfully used to meet energy deficiencies of cattle grazing WWP (Horn et al., 2005). Prior to being fed to cattle, grains generally undergo processing. The objective of processing grain is to optimize starch or energy availability by maximizing the extent of carbohydrate digestion in the rumen while controlling its rate of digestibility (Koenig et al., 2003). We hypothesized that microbial synthesis might be optimized when the rate of supplemental carbohydrate digestion is closer to the rate of ruminal fermentation of WWP. Therefore, the more rapid rate of ruminal fermentation of steam flaked corn (SFC) as compared with that of ground corn (GC) may be more appropriate for supplementation of cattle grazing WWP, which has a rapid rate of fermentation (Chabot et al., 2008). Therefore, the objective of this experiment was to evaluate effects of supplemental corn processing (ground versus steam-flaked) on intake, metabolizable protein, and digestibility of beef cows grazing WWP.

MATERIALS AND METHODS

All procedures and experimental protocols were approved by the New Mexico State University Institutional Animal Care and Use Committee. Eight Angus cross cows (587 + 49.0 kg) fitted with ruminal and duodenal cannulas were used in a complete randomized design. Cows were assigned randomly to 1 of 2 treatments: 1) supplemented with ground corn (**GC**), and 2) supplemented with steam-flaked corn (**SFC**). Supplement was offered at 0.4% of BW. An experimental period of 15 d consisted of an adaption period to grazing wheat pasture and supplement, consisting of d 1 through d 10, and a collection period consisting of d 11 through d 15. The experiment was conducted from March 23 through April 6, 2015. Cows grazed a single beardless winter wheat pasture (*Triticum hybernum*), with supplement offered individually once daily at 0900 h. Cows were gathered into a small holding pen in the corner of the wheat pasture, tied to the holding pen fence with halter and lead, and individually fed their allotted supplement. Cows were allowed access to their supplement for 30 min, after which the uneaten supplement was placed directly into their rumens through the ruminal cannula.

One day prior to the experimental period (d 10), ruminal evacuations were performed on 2 cows at 0900 h. Digesta was collected into plastic bags lining 133-L plastic containers before cows were allowed to return to pasture to graze for 60 min. Masticate samples were subsequently collected and dried in a 50ºC forced-air oven to a constant weight. Masticate samples were then ground in a Wiley mill

(model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen, and composited on an equal dry-weight basis. Gelatin capsules containing chromic oxide (8 g) were ruminally dosed twice daily (at 0700 h and 1900 h) on d 6 through d 15 of the experimental period to estimate fecal output which is required to calculate intake. Ruminal fluid samples were collected at 0 (prior to dosing), 3, 6, 9, 12, 15, and 21 h after dosing. Duodenal and fecal samples were collected during the collection period according to the following schedule: d 12, 0700 h and 1300 h; d 13, 0100 h and 1900 h; d 14, 1000 h, 1600 h, and 2200 h; d 15, 0400 h. Individual samples consisted of approximately 200 g (wet basis) of fecal material and 100 mL of duodenal chyme. Each sample type was composited for each cow for later analysis. At 0600 h on d 15 of the experimental period, 5 L of ruminal fluid was obtained and mixed with 5 L of saline solution (0.9% NaCl, wt/vol) for isolation of bacterial cells (Zinn and Owens, 1986). The ruminal fluid/saline mixture was cooled at 4°C for later bacterial isolation.

Laboratory Analysis. Duodenal, fecal, and bacterial samples were thawed, mixed, subsampled, dried in a freeze dryer (-50°C) for 96 h, and ground in a Wiley mill through a 2-mm screen. Duodenal, fecal, masticate, and supplement samples were analyzed for DM, ash, and CP (methods 930.15, 942.05, and 990.02, respectively; AOAC, 1997). Samples were additionally analyzed for NDF according to Van Soest et al. (1991) using an Ankom 200 fiber analyzer (Ankom CO, Fairport, NY). Concentration of Cr was determined in duodenal and fecal samples (Hill and Anderson, 1958), and duodenal samples were analyzed for purines (Zinn and Owens, 1986). Isolated ruminal bacteria were analyzed for DM, ash, N, and purines. Ruminal fluid/saline mixture was centrifuged at 1,000 x g for 10 min to remove protozoa and feed particle from the supernatant. The isolated bacteria were re-suspended in saline and centrifuged at 27,000 x g for 20 min. The final isolated bacteria were freeze-dried at 50°C for 96 h.

Calculations. Fecal and duodenal Cr concentration were used to calculate daily fecal DM output and chyme DM leaving the abomasum, respectively. Fecal DM output was determined by dividing Cr dose by fecal Cr concentration. Likewise, chyme DM leaving the abomasum was determined by dividing Cr dose by duodenal Cr chyme concentration. Supplement fecal DM output was calculated by multiplying supplement intake by supplement DM indigestibility, and forage fecal DM output was calculated by subtracting supplement fecal DM output from total fecal DM output. Forage DM intake was calculated as forage fecal output divided by forage in vitro indigestibility. Microbial OM and N leaving the abomasum were calculated using purines as a microbial marker (Zinn and Owens, 1986). Organic matter fermented in the rumen was considered equal to OM intake minus the difference between the amount of total OM reaching the duodenum and microbial OM reaching the duodenum. Feed N escape to the small intestine was considered equal to total N leaving the abomasum minus ammonia N and microbial N and, thus, includes endogenous N additions. Microbial N efficiency was calculated as g of duodenal microbial N per kg of OM fermented in the rumen.

Statistical Analysis. Data were analyzed as a completely randomized design with GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Effects were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Effects of GC or SFC supplementation on characteristics of ruminal and total tract digestion are shown in Table 1. Forage DM intake and total DM intake were greater ($P = 0.01$) for SFC than for GC supplementation. Forage OM, CP, and NDF intake were greater ($P = 0.01$) for SFC than for GC supplementation. Total OM, CP, and NDF intake were greater ($P \leq 0.02$) for SFC than for GC supplementation. Steam-flaked corn provided more energy at a faster rate for ruminal bacterial than GC due to its higher digestibility (Corona et al., 2006) and faster rate of digestion (May et al., 2009). Therefore, supplementation of SFC is more appropriate for cattle grazing WWP because the rate of digestion and rate of passage of WWP are very rapid (Chabot et al., 2008). Therefore, nutrients are better optimized in the rumen when SFC is supplemented as compared with GC to cattle grazing WWP.

Although feed CP flow to the small intestine was not affected ($P = 0.97$) by corn processing method, microbial CP synthesis was greater ($P = 0.01$) for SFC than for GC supplementation. Therefore, total CP flow to the small intestine (metabolizable protein) was greater ($P = 0.03$) for SFC than for GC supplementation. However, microbial efficiency was not affected ($P = 0.12$) by processing method. Total tract digestibility of OM, CP, and NDF (expressed as g/d) were greater ($P \leq 0.02$) for SFC than for GC supplementation. The greater nutrient digestibility for SFC than for GC and the lack of treatment effects on microbial efficiency suggests that the increased forage intake was a result of the improved digestibility of forage caused by SFC as compared with GC. Forage intake of cattle is regulated by signals of distention of the reticulo-rumen (Balch and Campling, 1962). Increasing digestibility subsequently decreases rumen volume and distention thereby allowing greater intake (McCollum and Galyean, 1985).

IMPLICATIONS

Results from this experiment imply that steam flaking as compared to grinding corn increases wheat pasture digestibility, subsequently increasing forage intake and microbial protein synthesis. Therefore, improved performance is expected with supplementation of steam-flaked corn as compared with supplementation of ground corn of cattle grazing WWP.

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Table 1. Effects of processing of supplemental corn on intake and characteristics of digestion of cows grazing wheat pasture

	Corn processing			
Item	Ground corn	Steam flaked corn	SE	*P*-value
DM intake, kg/d				
Forage	9.86	11.64	0.365	0.01
Supplement	2.13	2.13	0.096	0.97
Total	11.98	13.78	0.351	0.01
OM intake, kg/d				
Forage	8.18	9.66	0.302	0.01
Supplement	2.09	2.11	0.094	0.88
Total	10.27	11.77	0.292	0.01
CP intake, kg/d				
Forage	1.67	1.99	0.062	0.01
Supplement	0.19	0.16	0.007	0.04
Total	1.88	2.15	0.060	0.02
NDF intake, kg/d				
Forage	5.48	6.47	0.202	0.01
Supplement	0.31	0.29	0.013	0.27
Total	5.79	6.76	0.199	0.01
Flow to the duodenum, kg/d				
OM	5.47	5.67	0.351	0.71
NDF	1.56	1.74	0.123	0.33
CP				
Microbial	0.81	1.02	0.026	0.01
Feed	0.83	0.83	0.053	0.97
Total	1.64	1.86	0.056	0.03
Microbial efficiency[a]	24.19	29.14	1.926	0.12
Fecal output, kg/d				
OM	2.44	2.72	0.078	0.05
CP	5.50	6.03	0.021	0.12
NDF	1.42	1.75	0.060	0.01
Total tract digestibility, % of intake				
OM	76.22	76.91	0.245	0.09
CP	70.69	72.03	0.676	0.21
NDF	75.48	74.07	0.40	0.05
Total tract digestibility, g/d				
OM	782.1	9052.4	220.28	0.01
CP	1328.1	1552.1	47.07	0.02
NDF	4371.9	5008.9	147.22	0.02

[a]Grams of microbial N/kg of organic matter fermented

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Does adaptive grazing management influence dietary quality of yearlings during the grazing season on western Great Plains rangelands?[1]

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ABSTRACT: Grazing management decisions, such as timing of herd movements, can have a direct impact on the diet quality and nutritional plane of cattle. The variation in diet quality relative to adaptive versus continuous grazing strategies can lead to differences in cattle weight gains which directly impacts the profit margin for livestock producers. Near Infrared Reflectance Spectroscopy (NIRS) was used on fecal samples collected weekly from yearlings during the 2015 grazing season (May-October) to evaluate if differences occurred in measurements of dietary quality (crude protein and digestible organic matter) between adaptive grazing and continuous, season-long grazing in 2 rangeland ecosystems of the western Great Plains: shortgrass steppe and northern mixed-grass prairie. Yearling cattle under traditional grazing management at a moderate stocking rate had a 1.2 to 2.4% higher dietary crude protein ($p < 0.003$, $p < 0.001$) and a 0.5 to 1.4% higher digestible organic matter ($p < 0.1$, $p < 0.001$) than yearling cattle under adaptive grazing management across the season at HPGRS and CPER respectively, with maximum differences for both protein and digestibility exceeding 5% at times. At CPER, adaptive grazing management caused a 2 to > 4 fold steeper decline in digestibility between rotations compared to traditional grazing management.

Keywords: beef cattle, diet quality, grazing management

INTRODUCTION

Grazing management decisions directly impact herd nutrition and diet quality of cattle (Holechek et al. 1995). Functionally, this can positively or negatively affect livestock weight gain because, as stocking rate increases, the gain per head decreases but total livestock pounds produced per unit land area increases (Bement, 1969; Manley et al., 1995; Hart and Ashby, 1998; Derner et al., 2008). Ranchers use different grazing management strategies to attain their desired management goals (Roche et al., 2015). Adaptive grazing management (AGM) utilizes monitoring-informed decision-making to achieve desired management goals. We compared this contemporary grazing strategy to traditional grazing management (TGM), continuous season-long grazing, at moderate stocking rates in 2 rangeland ecosystems of the western Great Plains: shortgrass steppe and northern mixed-grass prairie. Arguments have been made for both systems, but the literature is inconclusive as to which grazing strategy is superior (Briske et al., 2008). This debate has yet to be resolved, but it is clear that stocking rate is one of the most important influences on plant and animal responses to grazing (Briske et al., 2008). Our aim was to determine if the adaptive grazing management strategy differed in dietary quality of yearlings across the summer grazing season (May-late September). Near Infrared Reflectance Spectroscopy (NIRS) was used on fecal samples collected weekly from yearlings to evaluate crude protein and digestible organic matter. NIRS is a non-invasive procedure that has proven highly effective in evaluating diet nutritional plane and quality (Holechek et al., 1982).

MATERIAL AND METHODS

Experimental Design and Treatments

Site Descriptions

Grazing experiments were applied in two semiarid rangeland ecosystems in the western Great Plains: (1) shortgrass steppe at the United States Department of Agriculture - Agricultural Research Service (USDA - ARS) Central Plains Experimental Range (CPER) (40°50'N, 104°43'W) approximately 12 km northeast of Nunn, Colorado, and (2) northern mixed-grass prairie at the USDA - ARS High Plains Grasslands Research Station (HPGRS) (41°11' N, 104°53' W) approximately 7 km northwest of Cheyenne, Wyoming. Mean annual precipitation is 340 mm and 381 mm at CPER and HPGRS,

[1]We would like to thank the Crow Valley Livestock Cooperative, Inc. for providing yearlings at the Central Plains Experimental Range, the King Ranch for providing yearlings at the High Plains Grasslands Research Station, and the USDA-ARS research technicians for their assistance in data collection

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respectively. Vegetation production is dominated by C_4 perennial grasses at CPER (Lauenroth and Burke, 2008) whereas production is co-dominated by both C_3 and C_4 perennial grasses at HPGRS (Manley et al., 1995; Derner and Hart, 2007). The dominant C_4 perennial grass species at both sites is blue grama [*Bouteloua gracilis* (Wild ex. Kunth) Lag. ex. Griffiths]. The dominant C_3 perennial grass species are needle and thread [*Hesperostipa comata* (Trin & Rupr.) Barkworth] and western wheatgrass [Pascopyrum smithii (Rydb.) A. Löve].

Grazing Strategies

CPER had two grazing strategies: AGM or adaptive grazing management (high stock intensity of yearling cattle, rotated among pastures across the grazing season) and TGM or traditional grazing management (continuous, season-long). For each of ten pairs of 129.5 ha pastures, one pasture was randomly assigned the AGM strategy and the other pair was assigned the TGM strategy. Pastures were paired based on similarity of ecological sites and pasture-level topographical wetness index (TWI) (USDA Adaptive Grazing Management Plan 2014). For both grazing strategies, stocking rate was the same (0.6AUM/ha), but the AGM strategy had a ten-fold greater stocking density (1.2 AU/ha compared to 0.12 AU/ha in the TGM) (USDA Adaptive Grazing Management Plan 2014). The planned rotational grazing sequence in the 10 pastures for AGM included 2 rested pastures and 8 pastures to be grazed, with movement of yearling cattle to the next pasture in the grazing sequence determined when a vegetation threshold of residue was attained: 336 kg/ha for pastures dominated by loamy ecological sites, 449 kg/ha for pastures with a mix of ecological sites, and 505 kg/ha for pastures dominated by sandy ecological sites). Due to well-above May precipitation in 2015 (2.5-fold greater than long-term average), vegetation thresholds were attained much slower than anticipated due to substantial forage production, and as a result, only 4 of the planned 8 grazed pastures were used. Movement between pastures occurred on May 27 (week 2), July 7 (week 8), and August 20 (week 14).

At HPGRS we had two similar grazing treatments with two replications, AGM1, AGM2, TGM1, TGM2. Stocking rates for AGM and TGM were 1.24 AUM/ha and 0.93 AUM/ha, respectively. Stocking rate was 29% greater in the AGM strategies due to extra remaining forage residue from the prior growing season. Cattle in the TGM treatment stayed in their pasture throughout the entire grazing treatment from early June to late September. The AGM herds were moved as determined by management. AGM1 herd was moved on 10 July (week 5), 27 July (week 8), and 14 August (week 10) 2015. AGM2 herd was moved on 22 June (week 3), 8 July (week 5), and 14 August (week 10) 2015. Both AGM1 and AGM2 were combined in one pasture on 14 August (week 10) 2015.

Assessing dietary quality

To assess dietary quality between the two grazing strategies in each rangeland ecosystem, we collected weekly fecal samples throughout the grazing season. At CPER, herd composite samples were collected from the AGM pasture being grazed and its paired TGM pasture. For the AGM strategy, the herd composite sample was combined from ten distinct steer fecal deposits. For the TGM strategy, the herd composite was combined from four distinct steer fecal deposits. At HPGRS, herd composites were taken weekly from both the TGM and AGM strategies as well. Two fecal subsamples were taken from each replication and combined to create one herd composite per treatment (n=4). All HPGRS herd composites consisted of four separate fecal deposits per treatment with a total of one herd composite/treatment/week.

Samples were sent to Texas A&M GANLAB for Near infrared Reflectance Spectroscopy (NIRS) to quantify fecal crude protein and digestible organic matter (DOM) (Holechek et al., 1982; Lyons and Stuth, 1991; Lyons and Stuth, 1992; Lyons et al., 1993; Tolleson and Schafer, 2014).

Statistical Analysis

Means and standard errors of each diet quality response variable were calculated using week of the grazing season as the replicate. Analysis of variance (ANOVA) was used to compare means between grazing strategies for each rangeland ecosystem at the 90% and 95% confidence levels using paired t-tests. Nutritional plane heterogeneity across the grazing season was determine for the grazing strategies by calculating the standard deviation for each dietary response variable. Partial linear least squares regression was calculated for crude protein and DOM within each rotation phase (i.e., time within a single pasture) to calculate the slope of a fit trendline as an indication of the decline in dietary quality.

RESULTS AND DISCUSSION

Crude Protein Results

At CPER, crude protein values across the grazing season were greater in the TGM strategy compared to the AGM strategy (mean ± standard error, 9.72 ± 0.55 % and 7.37 ± 0.49 %, $p < 0.001$) (Table 1). Crude protein levels between the AGM and paired TGM pastures started at very different levels, with AGM having a > 4 % lower crude protein level the very first week on pasture (Table 1). Rotation of the yearling steers in the AGM strategy to a new pasture increased crude protein levels from prior week measurements, but the levels were still less than the TGM strategy (Table 1).

Although yearlings in the AGM strategy exhibited a lower dietary crude protein level across the grazing season, this strategy did result in a more homogenous plane of nutrition for the steers (standard deviations = 2.19 and 2.47 for the AGM and TGM strategies, respectively), ($p > 0.8$) (Table 3). The AGM treatment DOM rate of decline

was found to be significantly lower than the TGM treatment ($p < 0.02$) (Table 4).

Similar to the shortgrass steppe, results from the northern mixed-grass prairie (HPGRS) indicate that crude protein levels for yearlings in the TGM strategy were significantly higher than the AGM strategy across the grazing season (8.91 ± 0.49 % and 7.69 ± 0.50 %, $p < 0.003$). Heterogeneity of the nutritional plane for yearlings using crude protein values was similar between grazing strategies (Standard deviations = 1.98 and 1.96 respectively) (Table 1).

Digestible Organic Matter Results

At CPER, DOM was lower for the AGM than the TGM strategy across the grazing season (61.0 ± 0.36 % and 62.4 ± 0.28 %, $p < 0.001$) (Table 2). This implies that yearling steers in the TGM strategy exhibited more selectivity in foraging, likely attributable to the lower stocking density.

At HPGRS, DOM was also greater for yearlings in the TGM vs. the AGM strategy across the grazing season ($p < 0.1$). Again, greater expression of selectivity by yearlings in the TGM strategy is likely responsible for this result.

For both rangeland ecosystems in the western Great Plains, the TGM strategy resulted in a higher nutritional plane for yearlings across the grazing season. Rotation of yearlings among pastures in the AGM strategy did not result in increased crude protein or DOM levels in either ecosystem. At HPGRS, slopes of dietary values did not differ between grazing strategies, (Table 4), but at CPER, DOM declined more rapidly with the AGM strategy as the grazing period lengthened within a pasture. Slopes were 2- to > 4-fold greater in AGM than TGM with higher r^2 values (Table 5). These negative responses in DOM values for yearlings appear to be related to greater among-animal competition and selection of plant parts higher in lignin and cellulose with the AGM strategy as the grazing period lengthened.

IMPLICATIONS

Understanding how grazing strategies influence dietary crude protein and DOM levels in yearling cattle provides producers with science-based information to improve decision-making. In the 2 rangeland ecosystems of the western Great Plains (shortgrass steppe and northern mixed-grass prairie), the AGM strategy consistently reduced crude protein digestible organic matter levels across the grazing season, resulting in reduced individual animal weight gain. However, this grazing strategy provides flexibility for the land manager to attain other desired management goals (e.g., greater between pasture vegetation heterogeneity) as well as a drought management insurance with the rested pastures that could be used if dry conditions exist. In addition, greater weight gains per *grazed* ha with the AGM strategy, provides capacity for the ranch to potentially capitalize on possible economic returns from a suite of ecosystem services (e.g., grassland bird habitat).

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Table 1. Weekly crude protein levels for the adaptive grazing management (AGM) and traditional grazing management (TGM) at the Central Plains Experimental Range (CPER) near Nunn, Colorado and AGM and TGM at the High Plains Grasslands Research Station (HPGS) located near Cheyenne, Wyoming in 2015.

Week	CPER Crude Protein (%)			HPGRS Crude Protein (%)		
	TGM	AGM	ΔTrt[1]	TGM	AGM	ΔTrt[1]
1	11.98	7.69	4.29	11.06	10.82	0.24
2	11.5	8.12	3.38	12.47	9.55	2.92
3	13.97	10.93	3.04	10.61	8.07	2.54
4	12.61	10.55	2.06	9.47	7.81	1.66
5	13.48	9.03	4.45	10.61	6.91	3.7
6	10.73	9.86	0.87	9.41	8.65	0.76
7	11.19	7.82	3.37	9.96	9.07	0.89
8	10.17	8.27	1.90	8.75	7.08	1.67
9	9.5	7.69	1.81	8.96	6.74	2.22
10	10.95	8.11	2.84	8.49	6.74	1.75
11	8.39	7.37	1.02	7.70	8.77	-1.07
12	9.15	7.22	1.93	9.78	10.71	-0.93
13	8.73	7.00	1.73	7.99	8.41	-0.42
14	9.18	6.98	2.20	6.53	4.39	2.14
15	10.51	9.26	1.25	5.35	4.39	0.96
16	7.92	7.42	0.50	5.53	4.99	0.54
17	7.06	6.49	0.57			
18	5.74	4.98	0.76			
19	6.75	4.65	2.10			
20	4.93	3.3	1.63			
Mean	9.72	7.37	2.09	8.91	7.69	1.22
SE	0.55	0.49	0.26	0.49	0.50	0.34
SD	2.47	2.19	1.16	1.96	1.98	1.36

[1]ΔTreatment = difference between TGM and AGM in crude protein
*underline indicate pasture movements of the AGM treatment

Table 2. Weekly digestible organic matter (DOM) levels for the adaptive grazing management (AGM) and traditional grazing management (TGM) at the Central Plains Experimental Range (CPER) near Nunn, Colorado and AGM and TGM at the High Plains Grasslands Research Station (HPGRS) located near Cheyenne, Wyoming in 2015.

Week	CPER DOM (%)			HPGRS DOM (%)		
	TGM	AGM	ΔTrt[2]	TGM	AGM	ΔTrt[2]
1	63.96	64.94	-0.98	64.37	64.35	0.02
2	62.44	63.03	-0.58	63.89	64.43	-0.54
3	63.90	63.79	0.11	63.16	62.51	0.65
4	63.72	61.99	1.73	62.92	61.57	1.35
5	64.11	60.80	3.31	63.81	61.44	2.37
6	65.15	60.09	5.06	62.62	61.33	1.29
7	62.79	59.33	3.46	62.53	62.07	0.46
8	62.77	61.14	1.63	61.51	60.92	0.59
9	62.03	62.07	-0.04	61.82	59.90	1.92
10	62.08	61.91	0.17	60.37	58.88	1.49
11	61.66	60.67	0.99	59.44	61.69	-2.25
12	61.57	60.89	0.68	61.62	61.07	0.55
13	61.28	60.03	1.25	61.53	61.23	0.3
14	61.68	59.57	2.11	60.64	60.61	0.03
15	63.22	61.61	1.61	59.68	60.61	-0.93
16	62.07	60.55	1.52	61.47	60.83	0.64
17	60.29	60.71	-0.42			
18	60.97	60.56	0.41			
19	61.42	58.50	2.92			
20	61.49	58.60	2.89			
Mean	62.43	61.04	1.39	61.96	61.47	0.50
SE	0.28	0.36	0.35	0.37	0.35	0.28
SD	1.25	1.62	1.56	1.48	1.42	1.13

[2]ΔTreatment = difference between TGM and AGM in digestible organic matter
*underline indicate pasture movements of the AGM treatment

Table 3. Comparison of the nutritional dietary crude protein (%) trendline decline of the adaptive grazing management (AGM) and traditional moderate continuous-season long grazing management (TGM) specific to each pasture movement.

Pasture Rotations		CPER linear regression crude protein	
		TGM	AGM
Phase 1	Slope	-0.1083	-0.0864
	r^2	0.8011	0.7903
Phase 2	Slope	-0.0265	-0.0279
	r^2	0.1524	0.6992
Phase 3	Slope	-0.1297	-0.1567
	r^2	0.8118	0.9747

Table 4. Comparison of the nutritional digestible organic matter (DOM, %) trend-line decline of the adaptive grazing management (AGM) and traditional moderate continuous-season long grazing management (TGM) specific to each pasture movement.

Pasture Rotations		CPER linear regression DOM (%)	
		TGM	AGM
Phase 1	Slope	-0.0303	-0.0932
	r^2	0.189	0.5851
Phase 2	Slope	-0.0164	-0.0716
	r^2	0.5179	0.8862
Phase 3	Slope	-0.0403	-0.0838
	r^2	0.297	0.8167

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Long-term progesterone influence on feed efficiency, body composition, non-esterified fatty acids and metabolic hormones in mature Rambouillet ewes[1]

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ABSTRACT: The objectives of this study were to evaluate the effects of long-term progesterone (P4) treatment on changes in feed efficiency, BW, body composition, NEFA and metabolic hormones in mature Rambouillet ewes. Thirty, multiparous, 5- and 6-yr-old Rambouillet ewes were stratified by age and metabolic BW and assigned randomly to receive long-term P4 administration using a sequential replacement of either a P4-containing controlled internal drug release device (CIDR) or non-P4-containing CIDR (CIDRX). Initially, ewes were synchronized for estrus using a 7 d CIDR and $PGF_{2\alpha}$ protocol. All ewes exhibited estrus within 72 h after $PGF_{2\alpha}$. Twelve d after estrus (d = 0), each ewe received either a CIDR (n = 15) or a CIDRX (n = 15). Every 14 d thereafter, the CIDR or CIDRX was removed from each ewe and replaced with a new CIDR or CIDRX for 126 d. Jugular venous blood samples were collected from each ewe at the time of CIDR or CIDRX replacement. Serum samples were assayed for P4, NEFA, insulin (INS), triiodothyronine (T3) and thyroxine (T4). Individual feed intake was recorded using GrowSafe units, beginning at d 0 following a 3-wk adaptation period. Ewes were fed a mixed grass hay diet ad libitum that met the nutrient requirements for maintenance. BW for each ewe was collected every 14 d when CIDR or CIDRX were replaced. Back fat (BF) and rib-eye area (REA) were measured for each ewe every 28 d using ultrasonography. BW, residual feed intake, BF and REA did not differ ($P > 0.10$) between CIDR- and CIDRX-treated ewes. Calculated estimates of body composition did not differ ($P > 0.10$) between CIDR- and CIDRX-treated ewes. NEFA, T3 and T4 concentrations did not differ ($P > 0.10$) between CIDR- and CIDRX-treated ewes. However, INS concentrations did differ ($P < 0.05$) between CIDR- and CIDRX-treated ewes. In conclusion, long-term P4 treatment did not appear to alter feed efficiency and partitioning of nutrients. However, maintaining P4 may alter the homeostatic relationship between INS and carbohydrate metabolism in ewes.

Key words: carcass traits, ewe, metabolism, progesterone, residual feed intake

INTRODUCTION

We hypothesized that long-term maintenance of progesterone (**P4**) concentrations would alter feed efficiency by altering metabolic processes of ewes (Herrygers et al., 2015). This was based on the work of Swartz et al. (2014), who reported that the total kg of TDN consumed per ewe per kg of lamb born was 24% greater in Rambouillet ewes from lines selected high (**HL**) reproductive rate than in ewes selected for low (**LL**) reproductive rate. The only endocrinological difference between ewes of these lines was that systemic concentrations of P4 were greater in HL ewes than in LL ewes between 60 and 120 d of gestation. One could interpret these results to mean that the apparent increase in efficiency of nutrient utilization in HL ewes during gestation was the result of increased concentrations of P4 between d 60 and d 120 of gestation. Additionally, Parr et al., 1987 showed that the nutritional status of pregnant ewes can alter systemic P4 concentrations which in turn could influence alter metabolic process that impact maintenance of pregnancy.

Last year we reported that 70 d of long-term P4 was an insufficient interval of P4 treatment to alter feed efficiency, BW or estimates of body composition of ewes. This year we hypothesized that long-term, systemic P4 concentrations may be related to increased feed efficiency and changes in partitioning of nutrients over a 126-d period that more closely mimics length of sustained P4 concentraitons during pregnancy. Thus, the objectives of this study were to evaluate the effects of long-term P4 treatment, independent of the influence of the placenta and fetus, on changes in feed efficiency, BW, body composition, NEFA and metabolic hormones in mature Rambouillet ewes.

The hypotheses tested in this experiment were that feed efficiency, BW, back fat (**BF**), rib-eye area (**REA**), body composition, NEFA and metabolic hormones do not differ between Rambouillet ewes treated with a long-term P4 regimen, maintained with P4-containing, controlled internal drug release devices (**CIDR**), or ewes not treated with non-P4-containg CIDR (**CIDRX**) for 126 d.

MATERIALS AND METHODS

This experiment was conducted at the Montana State University Bozeman Area Research and Teaching Facility. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Agricultural Animal Care and Use Committee (Protocol No. 2014-AA12).

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Animals and Housing

Thirty, multiparous, 5- and 6-yr-old commercial Rambouillet ewes from the Montana State University, Red Bluff Research Ranch flock in Norris, Montana were used for this study. Additionally, two 2-yr-old crossbred Suffolk x Rambouillet and one 4-yr-old Rambouillet, sexually experienced, epididymectomized rams were used for detection of estrus.

At the beginning of the study, each ewe received an electronic identification ear tag that was used to record feed intake in GrowSafe units (GrowSafe Systems Ltd., Airdrie, AB, Canada). Ewes were housed in four open-shed pens (33 m x 11 m) each of which contained a GrowSafe unit.

Treatments

Before the beginning of the feeding trial adaptation period, individual BW were collected on two consecutive days and averaged. The average of the BW for each ewe was used to calculate individual metabolic BW (**MBW** = $BW^{0.75}$). At the same time, estimates of BF and REA were obtained by ultrasonography over the 12th rib of each ewe.

Ewes were stratified by age and MBW, then assigned randomly to one of two treatments. Treatments were: 1) long-term P4 maintenance using P4-containing CIDR (CIDR; n = 15) or 2) no long-term P4 maintenance using a non-P4-containg CIDR backbone (**CIDRX**; n = 15). To make the CIDR backbone, the outer, P4-containing silastic membrane was removed by slicing down the long axis of the CIDR with a scalpel blade and peeling the membrane from the plastic T-shaped backbone. All CIDR backbones were soaked in 80% ethanol (vol/vol H_2O). They were dried and coated with three layers of Flex Seal Liquid Rubber (Swift Response, Weston, FL, USA) in order to minimize the abrasive properties of the backbone on the vaginal wall of ewes.

In this experiment it was necessary to normalize the length of the long-term P4 treatment to estrus of the estrous cycle of each ewe. This was accomplished using a modified 7-d CIDR and $PGF_{2\alpha}$ protocol (Abecia et al, 2011). Each ewe received a CIDR for 7 d. On d 7, CIDR were removed and each ewe was injected (i.m) with 12.5 mg of $PGF_{2\alpha}$ (dinoprost tromethamine; ProstaMate®, Vedico, Inc., St. Joseph, MO, USA). Ewes were then exposed to epididymectomized rams that had painted briskets to mark the rumps of any ewe that exhibited estrus. All ewes showed estrus within 72 h after $PGF_{2\alpha}$. Twelve d after estrus each CIDR-treated and CIDRX-treated ewe received a CIDR or CIDRX, respectively. This event was the beginning of the feeding trial and d 0 of the experiment. Maintenance of long-term P4 concentrations in each ewe was accomplished by replacing a CIDR every 14 d with a new CIDR. The backbones of the CIDRX-treated ewes were replaced every 14 d with fresh CIDR backbones.

Blood Sampling

Blood samples were obtained by venepuncture of the jugular vein from each ewe every 2 wk along with a CIDR or CIDRX was replacement. Blood samples were placed on cooled at 4º C overnight, and serum was decanted into polypropylene tubes and stored at -20ºC until assayed for metabolites and hormones.

BW and Ultrasonography for BF and REA

Body weights of each ewe were collected on two consecutive days beginning on d 0 and every 14 d associated with the replacement of either a CIDR or CIDRX. The averages of the two consecutive BW were considered the BW for that day. Estimates of BF and REA were obtained by ultrasonography every 28 d beginning at d 0.

Feeding Intake

A 126-d trial was conducted in order to estimate feed efficiency of CIDR- and CIDRX-treated ewes using the GrowSafe feed intake system. Ewes were given ad libitum access to mixed grass hay, water, and mineralized salt blocks. The chemical composition is given in Table 1. The chemical composition of the mixed grass hay on an as fed basis met the NRC (NRC, 2006) nutrient requirements for maintenance of a 60 kg adult ewe. Ewes were allowed a 3-wk adaptation period where the feed bars were removed from the GrowSafe units so that multiple ewes could eat at the same time. At the beginning of the experiment feed bars were replaced to ensure accurate measurements of individual ewe feed intake.

Residual Feed Intake Calculations

Daily intakes were computed for each of the ewes from the feed intakes derived from the GrowSafe Data software. Days that had scale noise greater than 12% and with assigned feed disappearance less than 92% were not used for feed intakes. Average daily gain (kg/d) of individual ewes were modeled by linear regression of bi-weekly BW using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC, USA). The regression coefficients were used to compute the ADG, initial and final BW, and mid-test MBW as described by Lancaster et al. (2009). Expected feed intake (**EFI**) was modeled using PROC GLM by linear regression of DMI against the modeled mid-test MBW and ADG (Koch et al., 1963). The model used to estimate EFI was:

$$Y_i = \beta_O + \beta_1 ADG_i + \beta_2\, mid\text{-}test\ MBWi + \varepsilon_i$$

where Y_i is the DMI of the ewe, β_O is the regression intercept, β_1 is the partial regression coefficient of DMI on modeled ADG, β_2 is the partial regression coefficient of DMI modeled on mid-test MBW, and ε_i is the residual error in the DMI of the ewes. Residual feed intake (**RFI**) was calculated for each ewe as the difference between DMI and EFI.

Calculated Estimates of Body Composition

Estimates of muscle mass (kg) and intra-muscular fat

(kg) were calculated from BF, REA and BW based on regression equations reported by Silva et al. (2006) for mature ewes. Estimates of empty body weight (kg), and proportions of empty body weight dry matter (%), empty body weight fat (%), empty body weight protein (%), carcass weight (kg), carcass weight dry matter (%), carcass weight fat (%), and carcass weight protein (%) were calculated from BW based on regression equations reported by Sanson et al. (1993) for mature ewes.

Metabolite and Hormone Assays

Progesterone, thyroxine (**T3**) and triiodothyronine (**T4**) concentration were assayed using radioimmunoassay (**RIA**) kits (MP Biomedical, Costa Mesa, CA) validated for sheep serum. Insulin (**INS**), concentrations were assayed using an RIA kit (EMD Millipore, Darmstadt, Germany) validated for sheep serum. Intra-assay CVs for a pooled sample that contained 0.18, 44, 0.56 ng/mL for T3, T4 and INS, respectively were 9.6%, 3.6% and 4.7%, respectively. Intra- and inter-assay CVs for a pooled sample that contained 0.31 ng/mL of P4 were 12% and 2.1%, respectively.

Concentrations of non-esterified fatty acids (**NEFA**) were quantified with a commercially available enzymatic-colorimetric assay (HR Series NEFA – HR [2]., Wako Diagnostics, Richmond, VA) validated for sheep serum. Intra- and inter-assay CVs for a pooled sample that contained 0.21 mEq/L of NEFA were 5.1% and 3.7%, respectively.

Statistical Analyses

Data for BW, RFI, BF, and REA at 70 d were analyzed by ANOVA for completely randomized design using PROC ANOVA of SAS. The model included treatment (CIDR and CIDRX). Means from each analysis were separated using Bonferroni's adjustment.

Data for muscle mass (kg), intra-muscular fat (kg), empty body weight (kg), empty body weight dry matter (%), empty body weight fat (%), empty body weight protein (%), carcass weight (kg), carcass weight dry matter (%), carcass weight fat (%), and carcass weight protein (%) were analyzed by ANOVA using separate PROC MIXED models for repeated measures of SAS. The model included treatment (CIDR and CIDRX), day (ultrasound day), and the treatment by day interaction. Ewe within treatment was the subject and d of ultrasound was the repeated measure. Means were separated using Bonferroni's multiple comparison adjustment.

Data for P4, T3, T4, INS and NEFA concentrations, and the T3:T4 ration were analyzed by ANOVA using separate PROC MIXED models for repeated measures of SAS (SAS, Cary, NC). The model included treatment (CIDR and CIDRX), day (ultrasound day), and the treatment by day interaction. Ewe within treatment was the subject and d of ultrasound was the repeated measure. Means were separated using Bonferroni's multiple comparison adjustment.

RESULTS

Progesterone concentrations on d 0 differed ($P < 0.05$) between those ewes that received a CIDR and those that received a CIDRX (Figure 1). There was a treatment by day interaction ($P < 0.05$) for P4 concentrations over the 126- d experimental period (Figure 1). Progesterone concentrations in CIDR-treated ewes decreased by d 14 and remained constant until d 84 than increased and remained high from d 96 to d 126. Whereas, P4 concentrations remain the sane in CIDRX-treated ewes until d 98 and remain low (< 1.5 ng/mL) until d 126 (Figure 1).

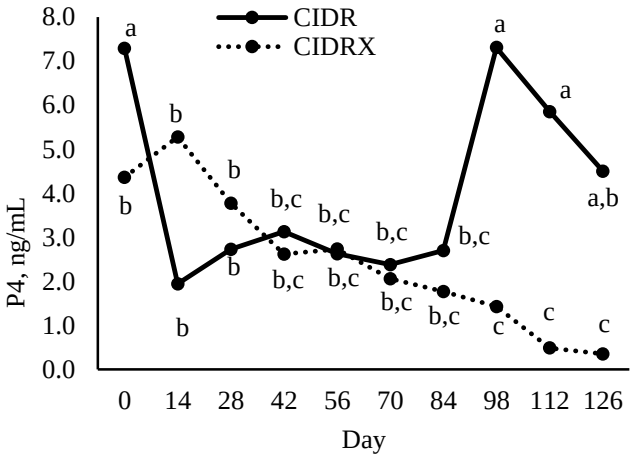

Figure 1. Progesterone (P4) concentrations at 14-d intervals in Rambouillet ewes given a P4-containing, controlled internal drug release devise (CIDR; n = 15) or a non-P4-containing CIDR (CIDRX; n = 15) beginning on d 12 (d 0 insertion of devises) of the estrous cycle relative to estrus. Interaction of treatment x d; $P < 0.05$. Different letters among points indicate differences at $P < 0.05$. Pooled SEM = 5.1 ng/mL.

Body weight, RFI, BF and REA did not differ between CIDR- and CIDRX-treated ewes (Table 2). Likewise, calculated estimates of muscle mass, intra-muscular fat, empty body weight, carcass weight; percentages of empty body weight as dry matter, fat, and protein; and, percentages of carcass weight as dry matter, fat, and protein did not differ between CIDR- and CIDRX-treated ewes (Table 3).

Non-esterified fatty acids, T3 and T4 concentrations did not differ between CIDR- and CIDRX-treated ewes (Table 4). However, NEFA concentrations differed ($P < 0.05$) between d 56 and d 126. Concentrations of T3 and T4 differed between d 84 and d 126 (Table 4). Concentrations of INS were greater ($P < 0.05$) in CIDRX-treated ewes than in CIDR-treated ewes and between d 0 and d 126 (Table 5).

DISCUSSION

To our knowledge this is the first study using CIDRs for maintenance of long-term P4. We were able to confirm that long-term P4 can be sustained using sequential replacement

of CIDRs. The difference between CIDRX- and CIDR-treated ewes on day 0 (d 12 of the estrous cycle) may be due to individual ewe differences in time of ovulation relative to estrus. Progesterone concentrations at d 14 in CIDRX ewes is consistent with a normal estrous cycle length and luteal function in the next cycle. Whereas, P4 concentrations is CIDR- treated ewes reflect early regression of the corpus luteum (Ottobre et al., 1980) and inhibition of estrus and ovulation. For CIDRX- treated ewes at d 42 represents the periovulatory period, which is characterized by low concentrations of P4. Thereafter, P4 concentrations in CIDRC- treated ewes continue to decrease from d 56 to d 126 as a result of the change in photoperiod associated with the onset of the anestrus season. This is reflected in a progressive increase in the proportion of anestrus ewes from 25% at d 56, 57% at d 84, and 95% at d 126.

On the other hand, P4 concentrations were maintained at ≥ 2 ng/ml between d 14 to d 84 as a result of P4 release from sequential replacement of CIDRs. In the study by Sarda et al. (1973), P4 concentrations in pregnant ewes did not markedly increase between d 80 and 90 of pregnancy. Furthermore, Swartz et al. (2014) reported that P4 concentrations did not differ between HL and LL ewes until after d 60. Our hypothesis for this study was that P4 concentrations needed to be maintained for longer than 70 d in order to cause a change in metabolism in sheep. In order to mimic pregnancy, two CIDRs were inserted at d 84 to increase P4 concentrations mimicking the change associated with pregnancy. Indeed, at d 98 P4 concentrations markedly increased indicating that two CIDRs do in fact increase P4 concentrations similar to those observed in pregnant ewes.

More importantly, the main focus of this study was to evaluate the effects of long-term P4 treatment on changes in feed efficiency, BW, body composition, metabolic hormones and NEFA in mature Rambouillet ewes, independent of placental and fetal functions. Mimicking pregnancy related changes in P4 concentrations in ewes using sequential replacement and number of CIDRs did not influence feed efficiency, BW, estimates of muscle mass, intra-muscular fat, empty body weight, carcass weight; percentages of empty body weight as dry matter, fat, and protein; and, percentages of carcass weight as dry matter, fat, and protein. Moreover, long-term P4 treatment did not affect T3, T4 or NEFA concentrations. However, it long-term P4 had lower concentrations of INS throughout the study. There is evidence that P4 increased INS resistance in rats (Kumagai et al., 1993), yet our results indicate that in sheep they are less I resistant at higher P4 concentrations.

In conclusion, it appears that maintaining P4 concentrations that mimic those during pregnancy for 126 d does not alter BW, feed efficiency, body composition, NEFA, T3 or T4. However, it appears that long-term P4 treatment may alter the mechanism associated with carbohydrate metabolism.

IMPLICATIONS

Our results indicate that systemic P4 concentrations are not directly related to increased feed efficiency and changes in partitioning of nutrients over a 126-d period that in pregnant ewes. However, maintaining P4 may alter the homeostatic relationship between insulin and carbohydrate metabolism.

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Table 1. Chemical composition of mixed grass hay diet[1]

Item	Mixed Grass Hay diet
Nutrient analyses	
DM	86.8
CP[2]	7.77
TDN[2]	59.5

[1] Ewes had free access to the mixed grass hay diet.
[2] CP and TDN are based on a percentage DM basis.

Table 2. Body weight (BW), residual feed intake (RFI), back fat depth (BF), and rib-eye area (REA) in Rambouillet ewes that received a P4-containing controlled internal drug release device (CIDR) or a non-P4 containing CIDR backbone (CIDRX) for 126 d

	Treatment			
Item	CIDR	CIDRX	SEM	*P*-value
n	15	15		
BW, kg	57.8	58.7	8.4	0.70
RFI, kg/d	-0.03	0.02	0.2	0.50
BF, mm	1.9	2.0	0.2	0.46
REA, mm^2	26.4	26.8	0.5	0.60

Table 3. Muscle mass (M), intra-muscular fat (IMF), empty body weight (EMW), empty body weight dry matter (EBWDM), empty body weight fat (EBWF), empty body weight protein (EBWP), carcass weight (CW), carcass weight dry matter (CWDM), carcass weight fat (CWF), and carcass weight protein (CWP) of Rambouillet ewes that received a P4-containing controlled internal drug release device (CIDR) or a non-P4 containing CIDR backbone (CIDRX) for 126 d

	Treatment			
Item	CIDR	CIDRX	SEM	*P*-value
n	15	15		
M, kg	13.3	13.7	2.2	0.60
IMF, kg	1.9	2.0	0.1	0.57
EMW, kg	48.9	49.1	2.9	0.92
EMWDM, %	45.4	45.5	0.4	0.92
EBWF, %	18.2	18.4	1.4	0.92
EBWP, %	17.82	17.79	0.05	0.92
CW, kg	26.9	27.1	1.0	0.92
CWDM, %	50.2	50.3	0.3	0.92
CWF, %	18.7	18.8	1.2	0.92
CWP, %	19.48	19.45	0.06	0.92

Table 4. Non-esterified fatty acids (NEFA), thyroxine (T3) and triiodothyronine (T4) concentrations of Rambouillet ewes that received a P4-containing controlled internal drug release device (CIDR) or a non-P4 containing CIDR backbone (CIDRX) for 126 d

	Day				
Item	0	28	56	84	126
n	30	30	30	30	30
NEFA, mEq/L[1]	0.35^{a,b}	0.37^{a,b}	0.27^{a}	0.37^{a,b}	0.43^{b}
T3, ng/mL	0.99^{a}	0.96^{a}	0.90^{a}	0.79^{b}	0.98^{a}
T4, ng/mL	38.9^{a}	38.5^{a}	35.2^{a}	30.7^{b}	35.7^{a}
T3:T4	0.025	0.025	0.026	0.026	0.028

^{a,b} Means within rows with different superscripts letters differ; $P < 0.05$.
^{1,2,3,4} Pooled SEM = 0.033 mEq/L; 0.05 ng/mL; 24.9 ng/m; 0.00004, for NEFA, T3:T4, and T3:T4 ratio, respectively

Table 5. Insulin concentrations of Rambouillet ewes that received a P4-containing controlled internal drug release device (CIDR) or a non-P4 containing CIDR backbone (CIDRX) for 126 d

	Treatment		
Item	CIDR	CIDRX	Mean[1]
n	15	15	
0	0.13	0.23	0.18^{a}
28	0.14	0.19	0.17^{a}
56	0.13	0.20	0.17^{a}
84	0.12	0.19	0.16^{a}
126	0.20	0.27	0.23^{b}
Mean[2]	0.14^{a}	0.22^{b}	

^{a,b} Means within a column or row with different letters differ; $P < 0.05$.
[1] Pooled SEM = 0.005 ng/mL.
[2] Pooled SEM = 0.003 ng/mL.

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Health evaluation of immune-stimulated and hay-supplemented feedlot receiving calves as assessed by blood gas analysis[1]

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ABSTRACT: This study evaluated blood parameters, health, and performance of immune-stimulated and hay-supplemented feedlot receiving calves. Heifers (n = 705; 179 ± 0.58 kg BW) were blocked by 6 truckloads and assigned to 48 pens and 4 treatments in a randomized complete block design. Treatments were a factorial arrangement of hay (+HAY vs. -HAY) and immunostimulation (+IMMUN vs. -IMMUN). Pens assigned +HAY received supplemental alfalfa hay to the receiving ration for the first 14 d. Calves assigned +IMMUN received a DNA immunostimulant on d 0. On d 0, 14, and 28, BW, rectal temperatures, and venous blood were collected. Health was recorded throughout the 56-d study, and pen weights on d 56. No HAY × IMMUN interactions occurred ($P \geq 0.18$). During the first 14 d, calf ADG was greater ($P < 0.01$) for +HAY than -HAY, but d 14 to 28 ADG was lower ($P < 0.01$) for +HAY than -HAY. Calf ADG was lower ($P \leq 0.01$) for +IMMUN than -IMMUN from d 28 to 56 and from d 0 to 56. Total DMI was greater ($P < 0.01$) for +HAY than -HAY from d 0 to 14, but lower ($P \leq 0.04$) from d 14 to 28 and from d 28 to 56. Gain efficiency of +HAY calves was greater (less negative; $P < 0.01$) from d 0 to 14, but lower ($P < 0.01$) from d 14 to 28 when compared to -HAY. Gain efficiency was lower ($P \leq 0.02$) for +IMMUN than -IMMUN calves from d 28 to 56 and d 0 to 56. Calf morbidity, mortality, and blood parameters (pH, glucose, lactate, hemoglobin saturated with oxygen [sO_2]) were not affected ($P \geq 0.18$) by treatments. Blood sO_2 was lower ($P < 0.01$) on d 0 than d 14 and 28, and glucose was greater ($P < 0.01$) on d 28 than d 0 and 14. Blood sO_2 correlated ($P < 0.05$) with glucose ($R^2 = 0.09$), lactate ($R^2 = -0.12$) and mortality ($R^2 = 0.08$). Glucose correlated with lactate ($R^2 = 0.61$), and first ($R^2 = -0.22$) and second ($R^2 = -0.13$) medical treatment. Lactate correlated ($P < 0.05$) with first medical treatment ($R^2 = -0.12$) and mortality ($R^2 = -0.12$). In conclusion, hay supplementation and immune stimulation did not affect calf health, performance or blood gas parameters. Changes in calf health can be observed in measures of blood parameters.

Key words: blood gas, calves, hay, immunostimulant.

INTRODUCTION

Bovine respiratory disease (**BRD**) accounts for 28.7% of all death and is the greatest health problem for newly received feedlot calves with estimated costs exceeding $692 million annually (NASS, 2006). Metaphylaxis with antibiotics and vaccination programs are currently industry standards for BRD control. However, it is anticipated that metaphylactic antibiotic use in food-producing animals will be restricted in the near future because of food safety and public health concerns.

Innate immune stimulation could alleviate the need for routine antibiotic use for BRD control in stressed receiving calves. Commercially available immunostimulants contain DNA plasmids resembling that of pathogenic bacteria and are capable of eliciting an innate immune response. In addition to immunostimulants and antibiotics, nutrition may alter the susceptibility of cattle to BRD. In particular, Rivera et al. (2005) reported a significant relationship between dietary roughage concentrations and feedlot calf morbidity.

Early detection of sick calves is critical for treatment of BRD, but current industry standards (visual observations and measurements of rectal temperature and respiration) for detecting BRD in cattle are ineffective. Blood oximetry values are used in human medicine to diagnose respiratory disease (pneumonia) and have been correlated to arterial hypoxia in cattle (Gebeyehu, 2010). Similar observations are expected for calves suffering from BRD. We hypothesize that receiving calves subjected to innate immunostimulation and hay-supplementation will have reduced susceptibility to disease, which we believe can be detected early by blood gas analysis. Therefore, the objective of our study was to evaluate blood gas parameters, health, and performance of immune-stimulated and hay-supplemented feedlot receiving calves.

MATERIALS AND METHODS

Animals and Facilities

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. A total of 705 crossbred heifers (179 ± 0.58 kg initial BW) shipped from southeastern Texas (1,159 km and 12 h in transit) were received in 6 truckloads that arrived 3 to 4 d apart. Upon arrival at the Clayton Livestock Research Center (Clayton, NM), calves rested for approximately 24 h with free access to two 132-L water fountains (CATTLEMASTER 840; Ritchie Inc., Conrad, IA), wheat

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hay, and 2 kg of a receiving diet (**RAMP**; Cargill Inc., Dalhart, TX). At initial processing (after the rest period), calves were individually weighed using a Daniels Bud Box System (Model AH-10; Ainsworth, NE), given a unique identification and pen tag, an oral de-wormer (Safe-guard; Intervet Inc., Merck Animal Health; Madison, NJ), and an anabolic growth implant (Ralgro, Merck Animal Health). All heifers were vaccinated with a modified live virus vaccine active against infectious bovine rhinotracheitis, bovine viral diarrhea type 1 and 2, parainfluenza 3 viruses, bovine respiratory syncytial virus, *Manheimia haemolytica* and *Pasteurella multocida* (Vista Once SQ; Intervet Inc., Merck Animal Health; Omaha, NE), and a bacterial vaccine against clostridial diseases (Vision 7, Intervet Inc., Merck Animal Health). No metaphylactic antibiotic treatment was used. Calves were housed in 48 soil-surfaced pens (12 m × 35 m; 11 m bunk space) for the 56-d experimental period with access to a 76-L water fountain (CATTLEMASTER 480; Ritchie Inc.) in each pen.

Experimental Design and Treatments

The experiment was a randomized complete block design with 48 pens (experimental unit) blocked by 6 truckloads of calves (116 to 120 calves per load). Within block, calves were randomly assigned to 8 pens (13 to 15 calves per pen), and pens of calves were randomly assigned to 4 treatments for a total of 12 pens per treatment across all blocks. Treatments were a factorial arrangement of supplemental hay (+HAY vs. -HAY) and DNA immune stimulation (+IMMUN vs. -IMMUN). All calves were fed RAMP for the 56-d experiment, and pens of calves assigned +HAY received alfalfa hay supplemented to the receiving ration on the opposite side of the feed bunk for the first 14 d (Table 1). Calves assigned to +IMMUN received a commercially available DNA immune stimulant (Zelnate; Bayer HealthCare LLC., Shawnee Mission, KS) via a 2-mL i.m. injection at initial processing. Indicated label use of this immunostimulant is to aid in the treatment of BRD associated with *M. haemolytica* during a perceived stressful event by stimulating the innate immune system. On d 14, all calves received 2 s.c. injections of booster vaccines matching the vaccines administered at initial processing (modified live virus vaccines and bacterial vaccine).

All cattle were fed the receiving diet once daily at 0700 using a feed truck with 6 individual bins, each fitted with a horizontal auger for dispensing feed. Pens of calves assigned to +HAY received hand delivered supplemental alfalfa hay once daily at 0930. Daily evaluations of unconsumed feed in bunks were made at approximately 0615, 1230 and 1830. Quantity of feed delivered was managed to allow for trace amounts of diet and hay in feed bunks at 1845 and no feed before the morning feeding (0700). This feed intake management procedure limited the amount of feed refusals. Representative samples of hay and receiving ration from every load delivered to the research facility were obtained for nutrient analysis by a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX).

Management and Collections

On d 0, 14, and 28, individual BW and rectal temperature (GLA M700; GLA Agricultural Electronics, San Luis Obispo, CA) measurements were recorded for all calves. On d 56, total group weights of pens of heifers were obtained using a livestock platform scale. Five calves within each pen were randomly selected for venous blood sample collection (via jugular venipuncture) at initial processing (d 0), and blood samples were collected from the same 5 calves on d 14 and 28. Calf health was assessed daily based on depression, anorexia, respiration, and temperature (**DART**; 3-point scale method). Calves showing signs of morbidity using the DART criteria were removed from their pens for further evaluation. Body weights and rectal temperatures were recorded and animals were given a severity score of 0 to 3 for depression and respiration. Based on these evaluations, calves warranted medical treatment if they had not gained weight since the previous recording, and (or) had a severity score of 2 or greater for depression or anorexia. A calf with a rectal temperature of 40.5°C or greater received medical treatment regardless of the severity score. First medical treatment consisted of a combination of antibiotic (florfenicol) and a fast-acting non-steroidal anti-inflammatory (flunixin meglumine) as a single dose (Resflor Gold; Merck Animal Health). The second medical treatment consisted of a tildipirosin solution, a semi-synthetic macrolide antibiotic (Zuprevo; Merck Animal Health). Calves requiring a third medical treatment were permanently removed from study and taken to the hospital facilities for further medical treatment.

Blood parameters of venous samples collected on d 0, 14, and 28 were analyzed using a benchtop blood gas analyzer (ABL815 FLEX; Radiometer America Inc., Westlake, OH). Parameters evaluated included pH, percentage of hemoglobin saturated with oxygen (**sO_2**), and blood glucose and lactate concentrations.

Table 1. Nutrient composition of receiving ration and hay

Nutrient[1]	RAMP[2]	HAY[3]
CP, % of DM	16.8	8.7
NE_m[3], Mcal/kg DM	1.72	0.82
NE_g[3], Mcal/kg DM	1.11	0.28

[1]Analyzed by Servi-Tech Laboratories (Amarillo, TX).
[2]Commercial feedlot receiving diet (Cargill Inc., Minneapolis, MN).
[3]Alfalfa hay.

Statistical Analysis

Performance and blood parameters were analyzed as continuous variables using the MIXED procedure (SAS Inst. Inc., Cary, NC), and morbidity and mortality were analyzed as categorical data using the GLIMMIX procedure (SAS Inst. Inc.). The statistical model included effects of HAY, IMMUN, and HAY × IMMUN, with block as random. Blood parameters were analyzed with day as repeated measure (covariance structure was compound symmetry). Correlations among blood parameters, morbidity, and mortality were analyzed using Pearson's

correlation (SAS Inst. Inc.). Differences were considered significant when $P \leq 0.05$ and considered as tendencies when $P \leq 0.10$.

RESULTS

Treatment Interactions. No HAY × IMMUN interactions occurred ($P \geq 0.20$) for calf BW, ADG, DMI, G:F, morbidity and mortality (Table 2). Similarly, no HAY × IMMUN interactions occurred ($P \geq 0.18$) for blood parameters (Table 3).

Effects of Hay. On d 14, BW of calves were greater ($P = 0.01$) for +HAY than -HAY (Table 2). Calf ADG was greater (less negative; $P < 0.01$) for +HAY than -HAY during the first 14 d, but was lower ($P < 0.01$) for +HAY than -HAY from d 14 to 28. Total DMI was greater ($P < 0.01$) for +HAY than -HAY from d 0 to 14, but lower ($P \leq 0.04$) from d 14 to 28 and from d 28 to 56. Gain efficiency (G:F) of +HAY calves was greater (less negative; $P < 0.01$) from d 0 to 14, but lower ($P < 0.01$) from d 14 to 28 compared to -HAY. Calf morbidity and mortality (Table 2), and blood parameters (Table 3) of calves were not affected ($P \geq 0.37$) by hay supplementation.

Effects of Immune Stimulation. On d 56, BW of calves were lower ($P = 0.01$) for +IMMUN that -IMMUN (Table 2). Calf ADG tended to be lower ($P = 0.08$) from d 14 to 28, and was lower ($P \leq 0.01$) from d 28 to 56 and d 0 to 56 for +IMMUN than -IMMUN calves. Total DMI tended to be lower ($P = 0.07$) for +IMMUN than -IMMUN calves from d 28 to 56. Gain efficiency (G:F) tended to be lower ($P = 0.10$) for +IMMUN than -IMMUN calves from d 14 to 28, and was lower ($P \leq 0.02$) from d 28 to 56 and d 0 to 56. Blood lactate (Fig. 1) was not different on d 0 and 14, but tended to be greater for +IMMUN than -IMMUN on d 28 (IMMUN × DAY, $P = 0.07$). Calf morbidity and mortality (Table 2), and blood parameters (Table 3) were not affected ($P \geq 0.29$) by immune stimulation.

Blood Parameters and Calf Health Correlations. Blood pH was correlated ($P < 0.05$) with sO_2 ($R^2 = 0.39$), glucose ($R^2 = -0.33$), lactate ($R^2 = -0.60$), and first ($R^2 = 0.09$) and second ($R^2 = -0.08$) medical treatment (Table 4). Blood sO_2 was correlated ($P < 0.05$) with glucose ($R^2 = 0.09$), lactate ($R^2 = -0.12$) and mortality ($R^2 = 0.08$). Glucose correlated with lactate ($R^2 = 0.61$), and first ($R^2 = -0.22$) and second ($R^2 = -0.13$) medical treatment. Lactate was correlated ($P < 0.05$) with first medical treatment ($R^2 = -0.12$) and mortality ($R^2 = -0.12$). Blood sO2 was lower ($P < 0.01$) on d 0 than d 14 and 28 (60.0 vs. 65.6 and 64.2 ± 0.99%), and glucose was greater ($P < 0.01$) on d 28 than d 0 and 14 (102.3 vs. 96.3 and 95.5 ± 1.84 mg/dL; results not included).

DISCUSSION

The hypothesis was that receiving calves subjected to hay supplementation and innate immune stimulation will be less susceptible to BRD. This could potentially be observed through greater performance and improved health (measured by morbidity and mortality) and blood gas parameters.

Effects of Hay. During the receiving period, increasing dietary roughage concentrations have been reported to decrease calf morbidity associated with BRD (Rivera et al., 2005). Challenges of managing stressed, newly received calves in a feedlot are to ensure adequate feed intake to meet energy requirements. Because ranch-weaned calves are not familiar with consuming a ration from feed bunks, hay supplementation is believed to encourage intake of calves in feedlot pens. However, greater DMI for +HAY than -HAY calves from d 0 to 14 was not enough to offset the energy dilution of the total diet with the addition of hay. Despite greater DMI, total NE_m intake of +HAY was lower than that of -HAY (3.30 vs. 3.60 Mcal/d), and neither met the calves NE_m requirements of 3.75 Mcal/d (NRC, 2000). Therefore, the potential benefits of hay supplementation on health were not observed possibly because of an energy deficiency.

With lower estimated energy intake for +HAY than -HAY calves from d 0 to 14, greater weight loss (greater negative ADG) was expected. In contrast, less weight loss (less negative ADG) was observed for +HAY than -HAY calves and could be explained by the effects of hay on gut fill. Gut fill tends to be greater with increased DMI, as well as for hay-based diets (Garza and Owens, 1989) due to increased salivation and water intake. Lower ADG for calves receiving +HAY from d 14 to 28 could be attributed to less energy available for gain due to lower DMI when compared to -HAY. Observed lower DMI after the hay supplementation period (after d 14) are possibly due to adaptation of the +HAY calves to a ration in feed bunks without supplemental hay.

Effects of Immune Stimulation. Innate immune stimulation could alleviate the need for antibiotic use by better preparing the immune system of stressed newly received calves. The pathogen associated genetic material contained within the immunostimulant is recognized by immune cell receptors which is indirectly responsible for the activation of cytokine genes. Cytokines then activate phagocytes and innate immune cells capable of destroying invading pathogen upon immune challenges (Tizard, 2009). However, prolonged glucocorticoid secretion as experienced during extended periods of stress could decrease cytokine production and result in inhibition of the innate immune responses (Carrol and Forsberg, 2007). Therefore, the lack of beneficial health responses to immunostimulation could be due to calves being subjected to extended periods of stress.

Lower ADG for +IMMUN than -IMMUN calves from d 14 to 28, and from d 28 to 56 coincides with greater blood lactate concentrations on d 28. The source of blood lactate is not clear, but it could have originated from diffusion through the rumen wall or from increased anaerobic metabolism in tissue. Increases in blood lactate originating from ruminal lactic acid have been reported to decrease DMI of cattle (Montgomery et al., 1963). Blood lactate has also been negatively associated with ADG in finishing beef cattle (Foote et al., 2016). Therefore, increased blood lactate on d 28 could, in part, be contributing to lower DMI and ADG from d 28 to 56.

Blood Parameters and Calf Health Correlations. The objective of this study was to evaluate calf health through blood gas parameters for immune-stimulated and hay-supplemented calves. Neither of the experimental treatments (immunostimulation and hay supplementation) significantly altered animal health (morbidity and mortality) or blood gas parameters. However, blood oximetry values for early detection of respiratory disease were correlated with individual animal health records.

Respiratory disease begins with the proliferation of pathogens and associated infection of the alveoli causing the pulmonary membranes to become inflamed and highly porous so that fluid, red blood cells and white blood cells pass out of the blood and into the alveoli (Guyton, 1991). Filled alveoli have decreased surface area available for gas exchange, compromising the ability of oxygen to pass into the blood and bind hemoglobin. Therefore, lower sO_2 (hemoglobin saturated with oxygen) levels are expected to be associated with BRD affected calves and were evident in the correlations observed between calf mortality and blood sO_2. Lower blood sO_2 levels on d 0 vs. d 14 and 28 could be associated with increased stress experienced on d 0. During initial processing, animals are subjected to a number of stress factors, which initiates a fight-or-flight response (Kovacs and Ojeda, 2012) increasing cellular metabolism and consequently the demand for O_2. Therefore, the lower venous O_2 on d 0 vs. d 14 and 28 could be explained by the increased O_2 demands of tissue during stress. Greater blood glucose levels on d 28 vs. d 0 and 14 possibly are a result of improved nutritional status.

Correlations between first medical treatment and blood pH, glucose, and lactate of calves could be attributed to nutritional status. Reduced feed intakes have been reported for BRD-affected morbid calves (Galyean and Hubbert, 1995). This suggests that calves on a higher plane of nutrition are less likely to be associated with BRD, and will potentially have greater blood glucose and lactate levels. In contrast to a negative correlation between blood lactate and first medical treatment, blood lactate was positively correlation to mortality. This positive correlation could be explained by increased anaerobic metabolism caused by a deficient supply of O_2 to tissue as a result of respiratory disease.

IMPLICATION

These results imply that hay supplementation and innate immune stimulation do not affect calf health, performance or blood gas parameters. Correlations between blood parameters and calf health suggest the possible application of blood gas analysis as a diagnostic tool for early detection of bovine respiratory disease.

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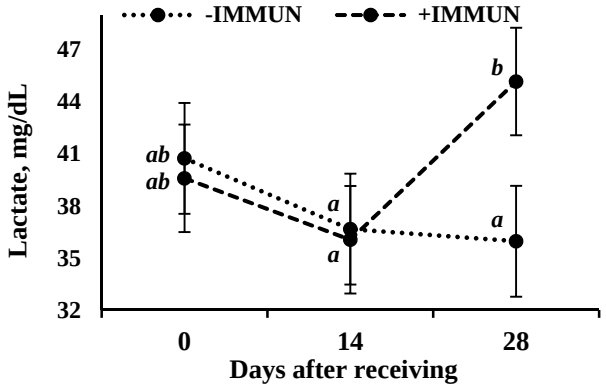

Figure 1. The effect of immunostimulation on blood lactate levels of heifers during the first 28 d after receiving. Effects were: IMMUN × DAY ($P = 0.07$), IMMUN ($P = 0.43$) and DAY ($P = 0.22$).

Table 2. Effects of immunostimulation and hay supplementation on health and performance of newly received heifers

	Treatments[1]					*P*-value		
	-HAY		+HAY					HAY ×
Item	-IMMUN	+IMMUN	-IMMUN	+IMMUN	SEM	HAY	IMMUN	IMMUN
BW, kg								
d 0	178.9	178.4	179.3	178.9	1.28	0.65	0.69	0.96
d 14	169.1	171.3	174.6	175.0	2.01	0.01	0.48	0.60
d 28	184.4	183.3	181.8	179.6	2.62	0.17	0.46	0.82
d 56	221.3	213.3	216.7	210.8	3.12	0.19	0.01	0.70
ADG, kg/d								
d 0 to 14	-0.70	-0.51	-0.34	-0.28	0.10	< 0.01	0.17	0.42
d 14 to 28	1.09	0.85	0.51	0.33	0.16	< 0.01	0.08	0.78
d 28 to 56	1.32	1.07	1.25	1.11	0.09	0.80	< 0.01	0.36
d 0 to 56	0.76	0.62	0.67	0.57	0.06	0.12	0.01	0.70
DMI, kg/d								
d 0 to 14	2.05	2.13	2.68	2.66	0.11	< 0.01	0.71	0.61
HAY	0	0	1.44	1.42	-	-	-	-
RAMP	2.05	2.13	1.23	1.24	-	-	-	-
d 14 to 28	4.04	4.10	3.67	3.52	0.16	< 0.01	0.76	0.48
d 28 to 56	5.74	5.49	5.45	5.13	0.17	0.04	0.07	0.82
d 0 to 56	4.28	4.24	4.26	4.05	0.13	0.41	0.32	0.49
G:F								
d 0 to 14	-0.36	-0.27	-0.13	-0.13	0.05	< 0.01	0.23	0.32
d 14 to 28	0.27	0.21	0.14	0.09	0.04	< 0.01	0.10	0.90
d 28 to 56	0.23	0.20	0.23	0.22	0.01	0.30	0.02	0.20
d 0 to 56	0.18	0.15	0.15	0.14	0.01	0.08	< 0.01	0.44
Morbidity[2], %								
M TRT 1[2], %	56.87	56.30	52.51	55.72	4.77	0.51	0.72	0.61
M TRT 2[3], %	26.63	28.91	22.51	27.20	3.82	0.37	0.29	0.68
Mortality[4], %	7.39	5.11	5.65	7.96	2.04	0.76	0.96	0.22

[1]Treatments were a factorial arrangement of supplemental hay (+HAY vs. -HAY) and DNA immune stimulation (+IMMUN vs. -IMMUN); 12 soil-surface pens with 13 to 15 calves per pen for each treatment.
[2]Percentages of calves receiving first (M TRT 1) and second (M TRT 2) medical treatments.
[3]Percentage of calves that died during the 56-d experimental period.

Table 3. Effects of immunostimulation and hay supplementation on venous blood parameters

	Treatments[1]					*P*-value[2]		
	-HAY		+HAY					HAY ×
Item[3]	-IMMUN	+IMMUN	-IMMUN	+IMMUN	SEM	HAY	IMMUN	IMMUN
pH	7.415	7.410	7.411	7.407	0.008	0.64	0.60	0.95
sO_2[4], %	62.8	63.0	63.1	64.3	1.37	0.58	0.61	0.72
GLC[4], mg/dL	97.2	101.0	98.4	95.6	2.5	0.40	0.84	0.18
LAC[4], mg/dL	35.6	41.8	39.9	38.8	3.2	0.83	0.43	0.24

[1]Treatments were a factorial arrangement of supplemental hay (+HAY vs. -HAY) and DNA immune stimulation (+IMMUN vs. -IMMUN).
[2]Effects of IMMUN × DAY interactions on venous blood parameters are reported in Fig. 1.
[3]Venous blood parameter analyzed with a bench top blood gas analyzer (ABL815 FLEX; Radiometer America Inc., Westlake, OH).
[4]Percentage of hemoglobin saturated with O_2,(sO_2), blood glucose (GLC), and blood lactate (LAC).

Table 4. Pearson's correlation coefficients among venous blood parameters, medical treatment, and mortality

	pH	sO_2	GLC	LAC	M TRT 1	M TRT 2	Mortality
pH[1]	1						
sO_2[1]	0.39*	1					
GLC[1]	-0.33*	0.09*	1				
LAC[1]	-0.60*	-0.12*	0.61*	1			
M TRT 1[2]	0.09*	-0.06	-0.22*	-0.12*	1		
M TRT 2[2]	0.08*	-0.03	-0.13*	-0.04	0.52*	1	
Mortality	-0.03	-0.08*	-0.02	0.10*	0.21*	0.37*	1

[1]Venous blood parameters analyzed with a benchtop blood gas analyzer (ABL815 FLEX; Radiometer America Inc., Westlake, OH).
[2]Percentages of calves receiving first (M TRT 1) and second (M TRT 2) medical treatments.
*Pearson's correlation coefficients ($P < 0.05$).

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Effect of post-weaning heifer development system on average daily gain, pregnancy rates, and subsequent feed efficiency as a pregnant heifer

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ABSTRACT: A 4-yr study was conducted using Angus-based, spring born heifers. In Yr 1, weaned heifers grazed corn residue (CR, n = 50) or were fed in a drylot (DLHI, n = 50). In Yr 2, 3, and 4, heifers grazed CR (n = 75), upland range (RANGE, n = 75), or were fed diets differing in energy, high (DLHI, n = 75) or low (DLLO, n = 75), in a drylot. Percent of mature BW prior to the breeding season was greater (P = 0.01) for DLHI (67%) compared with Range (59%), CR (60%), and DLLO (63%). Pregnancy rates to AI were similar (P = 0.39) among treatments (67, 63, 61, 49 ± 7.2%; RANGE, CR, DLHI, DLLO), and final pregnancy rates were also similar (84, 90, 91, 91 ± 5.4%; Range, CR, DLHI, DLLO; P = 0.59). A subset of AI pregnant heifers from each treatment was placed in a Calan gate system. Heifers were allowed a 20 d acclimation period before beginning the 90 d trial at approximately gestational d 170. Heifers were offered ad libitum hay; amount offered was recorded daily and orts collected weekly. Initial BW was not different (P = 0.35) among treatments (451, 457, 472, 464 ± 10 kg; RANGE, CR, DLHI, DLLO). Body weight at the end of the trial was also similar (P = 0.24; 488, 497, 511, 502 ± 14 kg; RANGE, CR, DLHI, DLLO). Intake was similar, either as DMI (P = 0.27; 9.74, 9.97, 10.18, 10.00 ± 0.76 kg; RANGE, CR, DLHI, DLLO) or residual feed intake (P = 0.61; 0.094, 0.091, -0.056, -0.0743 ± 0.160 kg; RANGE, CR, DLHI, DLLO). There was no difference in ADG (P = 0.36; 0.38, 0.45, 0.43, 0.41 ± 0.17 kg/d; RANGE, CR, DLHI, DLLO) among treatments. Although the development cost was not different among treatments (P = 0.41; $166, 141, 160, 171 ± 12, RANGE, CR, DLHI, DLLO), there was a $30 numerical difference between the most (DLHI) and least (CR) expensive treatment. Developing heifers to a greater pre-breeding BW did not influence subsequent AI or overall pregnancy rates or feed efficiency as a pregnant heifer.

Key words: beef heifers, feed conversion, heifer development

INTRODUCTION

Retaining and developing replacement heifers presents one of the largest expenses to the cow-calf producer, only surpassed by feed expense. Developing heifers to a lower target BW than previously recommended has been shown to reduce development cost, without any pregnancy rate reduction (Feuz, 1992; Clark et al., 2005). Previous research comparing corn residue and drylot systems has found heifers in the drylot gained more during the development period than on corn residue (Summers et al., 2014). However, heifers developed on corn residue experienced increased post AI ADG while on summer range, compared with heifers developed in confinement. It remains unclear if this difference is a result of compensatory gain or retained learned grazing behavior as suggested by Summers et al. (2014). Recently, greater effort has been made to select feed efficient animals. However, it remains unclear if selection for greater efficiency results in decreased DMI in the mature cow (Meyer et al., 2008). Understanding the long term effects of heifer development on cow efficiency will allow producers to make better management decisions. Whether a difference lies in behavioral effects as suggested by Summers et al. (2014) or previous diet quality, effects on mature cow intake as a result of development system have the potential to impact beef producers' profitability. Therefore, objectives of the current study were to determine if post-weaning heifer development system affected ADG, pregnancy rates, and subsequent feed efficiency as a pregnant heifer.

MATERIALS AND METHODS

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all procedures and facilities used in this experiment.

Post-Weaning Development

A 4-yr study conducted at the West Central Research and Extension Center (**WCREC**), North Platte, NE utilized crossbred, Angus-based, spring born heifers. In Yr 1, weaned heifers grazed corn residue (**CR**, n = 50) or were fed in a drylot (**DLHI**, n = 50). In Yr 2, 3, and 4, heifers grazed CR (n = 75), upland range (**RANGE**; n = 75), or were fed 1 of 2 drylot diets (Table 1) differing in energy, high (DLHI, n = 75) or low (**DLLO**, n = 75). Heifers developed on CR (n = 125) grazed corn residue from mid-November through mid-February and then grazed winter range. RANGE heifers (n = 125) grazed winter range during the treatment period. While grazing corn residue or winter range, heifers received the equivalent of 0.45 kg · hd^{-1} · d^{-1} of a 29% CP, dried distillers grain-based supplement containing monensin, with hay provided in times of deep snow. All heifers were managed together in a drylot during estrus synchronization and AI.

Table 1. Drylot diet composition (DM basis) offered to replacement heifers

Ingredient, %	DLHI[1]	DLLO[2]
Hay	74	83
Wet CGF	21	12
Heifer supplement[3]	5	5

[1] DLHI = heifers in Yr 1, 2, 3, and 4 received a high-energy diet in the drylot for 170 d.
[2] DLLO = heifers in Yr 2, 3, and 4 received a low-energy diet in the drylot for 170 d.
[3] Supplement = (81.35% of supplement, DM basis), limestone (11.11%), iodized salt (5.55%), trace mix (1.39%), Rumensin-90 (0.37%), and Vitamins A-D-E (0.22%).

Prior to estrus synchronization, 2 blood samples were collected 10 d apart via caudal venipuncture. Plasma progesterone concentration was determined via direct solid phase RIA (Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA) Heifers with greater than 1 ng/mL at either collection were considered pubertal. Heifers were synchronized using the melengesterol acetate-prostaglandin $F_{2\alpha}$ (**MGA-PG**) protocol. Heat detection aids (Estrotect, Rockway Inc., Spring Valley, WI) were applied at time of PG injection (Lutalyse, Zoetis, Florham Park, NJ). Heifers in standing estrus were AI 12 h later. Heifers not expressing estrus received a PG injection 6 d following the first PG injection and placed with bulls. Remaining heifers were combined with the non-AI heifers and bulls 10 d following AI on range at a 1:50 bull to heifer ratio for 60 d. Pregnancy diagnosis was conducted via transrectal ultrasonography (ReproScan, Beaverton, OR) 45 d following AI. Forty-five d after bull removal a second pregnancy diagnosis determined final pregnancy rate.

Pregnant Heifer Feed Efficiency

In mid-October, following pregnancy diagnosis, a subset of AI-pregnant heifers from each treatment (RANGE, n = 36; CR, n = 46; DLHI, n = 48; DLLO, n = 23) were placed in a Calan gate system. Heifers were allowed a 20 d acclimation period before beginning a 90 d trial at approximately gestational d 170. Heifers were offered ad libitum hay (7.9% CP); individual amounts offered were recorded daily and orts collected weekly.

Economic Analysis

Due to price fluctuations when the experiment was conducted, an average 5 yr price was used for economic analysis. Heifer value was obtained for the wk heifers were received (USDA-AMS, 2010a, 2011a, 2012a, 2013a, 2014a). Pasture values were calculated as half the cost of a cow-calf pair in the Southwest region of Nebraska and were obtained from the Nebraska Farm Real Estate Market Highlights (Johnson et al., 2010, 2011, 2013; Johnson and Van Newkirk, 2012; Jansen and Wilson, 2014). Wet corn gluten prices were obtained from the USDA-AMS for the third wk in September using Kansas City values (USDA-AMS, 2010b-2014b). Hay prices were also obtained for the third wk of September in the Platte Valley from the Nebraska and Iowa Hay report (USDA-AMS, 2010c-2014c). Actual supplement costs, both drylot and cube, were used. Other expenses include interest (6.5% of heifer value), vaccine, yardage, trucking for CR heifers, breeding expenses, and other miscellaneous expenses. Cull values of non-pregnant heifers were obtained for the wk of final pregnancy diagnosis (USDA-AMS, 2010d-2014d). The net cost of 1 pregnant heifer was calculated using the procedure defined by Feuz (1992). The value of 1, non-pregnant heifer was divided by 1 minus pregnancy rate to determine the value of cull heifers per pregnant heifer. This value was subtracted from the total development cost. Finally, the adjusted development cost is divided by pregnancy rate to determine the net cost of 1 pregnant heifer.

Statistical Analysis

Treatment was heifer development system in which CR and DLHI were replicated in 4 yr and RANGE and DLLO were replicated in 3 yr. Year was considered the experimental unit, with development treatment the fixed effect. Data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary NC). Pregnancy analyses included AI technician as a random effect. Pregnant heifer feed efficiency analyses included pen as a random effect. A P-value ≤ 0.05 was considered significant, with P-values between 0.05 and 0.1 considered tendencies. Least square means and SE of the proportion of pubertal and pregnant heifers by treatment were obtained using the ILINK function.

RESULTS AND DISCUSSION

Post Weaning Development Treatment (Table 2)

Heifers had a similar initial BW (P = 0.88, 235 ± 5 kg). During development, ADG was greater (P = 0.01) for DLHI heifers (0.71 ± 0.05 kg/d) compared with RANGE and CR (0.44 and 0.39 ± 0.05 kg/d, respectively). Differences in ADG resulted in a similar trend in post-treatment BW; DLHI heifers were heavier than RANGE and CR heifers (P < 0.01) but similar to DLLO heifers. At pre-breeding, percent of mature BW was greater (P = 0.01) for DLHI heifers compared with RANGE and CR heifers. Pubertal status prior to synchronization was similar (P = 0.20). Average daily gain following AI to the first pregnancy diagnosis was similar (P = 0.20) among treatments. Pregnancy rates to AI were similar (67, 63, 61, 49 ± 7.2%; RANGE, CR, DLHI, DLLO; P = 0.39), and final pregnancy rates were also similar (84, 90, 91, 91 ± 5.4%; Range, CR, DLHI, DLLO; P = 0.59). Although DLHI heifers had the lowest ADG following AI, BW at the first pregnancy diagnosis was greatest (P = 0.02) for DLHI heifers compared with other treatments. However, final pregnancy diagnosis BW was similar (P = 0.13). Furthermore, calving rate was greater (P = 0.04) for CR and DLHI heifers compared with RANGE heifers. Eborn et al. (2013) reported similar pregnancy rates for heifers

developed on high or low gain diets from 8 mo to d 21 of the breeding season. However, a larger proportion of high gain heifers became pregnant in the first 21 d of the breeding period, whereas in the current study the proportion of heifers that calved within the first 21 d was similar ($P = 0.24$).

Pregnant Heifer Feed Efficiency (Table 3)

In the feed efficiency trial, initial and final BW was similar ($P > 0.24$). Intake did not differ either as DMI ($P = 0.27$) or as residual feed intake (**RFI**; $P = 0.61$). There was no difference ($P \geq 0.30$) in ADG or G:F. Recent emphasis on genetic selection for feed efficient cattle to optimize profit in the feed yard has led to the idea of increased feed efficiency in the cow herd. Although potential exists for feed cost savings by increasing feed efficiency, reproductive performance could be compromised. Basarab et al. (2011) found heifers selected for high feed efficiency had lower pregnancy ($P = 0.09$) and calving ($P = 0.05$) rates than low efficiency contemporaries. In the current study, development treatment did not impact feed efficiency as a pregnant first calf heifer. Future studies investigating the impacts of heifer development system on lifetime feed efficiency are needed.

Economic Analysis (Table 4)

Heifers began development with the same value and receiving diet expense. Diet cost was different ($P < 0.01$) among treatments with the exception of RANGE and CR, which had similar ($P = 0.56$) treatment costs. The most expensive diet, DLHI, and the mean of the 2 least expensive diets, RANGE and CR, indicated a $41 difference. Summer pasture and additional expenses were similar across treatments. Due to numerical differences in pregnancy rates and BW at pregnancy diagnosis, cull heifer value was different ($P < 0.01$) among treatments where RANGE heifers, with the numerically lowest pregnancy rate, had the greatest cull heifer value. These data differ from previous studies that reported similar cull heifer value on intensive and extensive heifer development (Funston and Larson, 2011; Summers et al., 2014). Numerically higher final pregnancy rates resulted in lower cull value for DLHI and DLLO heifers. Net cost per pregnant heifer was similar ($P = 0.99$) among treatments using 5 yr average prices. This contrasts previously reported data suggesting extensive development reduced ($P = 0.01$) cost by $45 per pregnant heifer (Funston and Larson, 2011). Differences may be due to the extreme price fluctuation in the years this experiment was conducted.

IMPLICATIONS

In the current experiment, heifer development strategy did not impact AI or final pregnancy rates. Cost per pregnant heifer was similar among treatments. As a pregnant first calf heifer, feed efficiency was not impacted by development system. These results indicate producers may utilize their most readily available and/or cost-effective resources with no detriment to pregnancy rates or feed efficiency as first-calf heifers.

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Table 2. Effect of development system on heifer gain and reproductive performance

Item	RANGE[1]	CR[2]	DLHI[3]	DLLO[4]	SEM	*P*-value
n	75	125	125	75		
Initial BW, kg	234	236	235	234	5	0.88
Post-development BW[5], kg	301[b]	299[b]	346[a]	321[a,b]	8	< 0.01
Development ADG, kg	0.44[b]	0.39[b]	0.71[a]	0.57[a,b]	0.05	0.01
Pre-breeding BW, kg	324[b]	329[b]	372[a]	347[a,b]	9	0.01
Percent of mature, %	59[b]	60[b]	67[a]	63[a,b]	2	0.01
Pubertal status, %	28	41	86	77	10	0.20
Synchronization ADG, kg	0.71	0.81	0.69	0.78	0.11	0.20
AI pregnancy diagnosis BW, kg	364[b]	371[b]	396[a]	376[a,b]	6	0.02
Final pregnancy diagnosis BW, kg	427	427	447	432	11	0.13
Breeding ADG[6], kg	0.76[a,b]	0.80[a]	0.46[c]	0.57[b,c]	0.10	< 0.01
AI pregnancy, %	67	63	61	49	7	0.39
Final pregnancy, %	84	90	91	91	5	0.59
Calving rate[7], %	70[b]	87[a]	87[a]	86[ab]	7	0.04
Calved in first 21 d, %	77	69	65	55	12	0.24

[1] RANGE = heifers were offered the equivalent of 0.45 kg · hd^{-1} · d^{-1} while grazing winter range for 170 d before entering the drylot for estrus synchronization and AI.
[2] CR = heifers were offered the equivalent of 0.45 kg · hd^{-1} · d^{-1} while grazing corn residue for 90 d and winter range for 80 d before entering the drylot for estrus synchronization and AI.
[3] DLHI = heifers were developed in the drylot for 170 d and through estrus synchronization and AI on a high-energy diet.
[4] DLLO = in Yr 2, 3, and 4 heifers received a low-energy diet in the drylot for 170 d through estrus synchronization and AI.
[5] BW at the time of blood collection.
[6] ADG in the period between prebreeding and first pregnancy diagnosis.
[7] Percentage of heifers that calved.
[a,b,c] Means in a row with different superscripts are different ($P \leq 0.05$).
[x,y] Means in a row with different superscripts are different ($0.05 \leq P < 0.1$).

Table 3. Effects of heifer development system on pregnant heifer feed efficiency

Item	RANGE[1]	CR[2]	DLHI[3]	DLLO[4]	SEM	*P*-value
n	36	46	48	23		
Initial BW, kg	451	457	472	464	10	0.35
Mid BW, kg	468	477	492	482	9	0.25
Final BW, kg	488	497	511	502	14	0.24
DMI, kg	9.74	9.97	10.18	10.00	0.76	0.27
ADG, kg	0.38	0.45	0.43	0.41	0.17	0.36
RFI	0.094	0.091	-0.056	-0.074	0.160	0.61
G:F	0.039	0.045	0.042	0.041	0.016	0.30

[1] RANGE = heifers were offered the equivalent of 0.45 kg · hd^{-1} · d^{-1} while grazing winter range for 170 d before entering the drylot for estrus synchronization and AI.
[2] CR = heifers were offered the equivalent of 0.45 kg · hd^{-1} · d^{-1} while grazing corn residue for 90 d and winter range for 80 d before entering the drylot for estrus synchronization and AI.
[3] DLHI = heifers were developed in the drylot for 170 d and through estrus synchronization and AI on a high-energy diet.
[4] DLLO = heifers received a low-energy diet in the drylot for 170 d through estrus synchronization and AI.

Table 4. Economic analysis (5 yr avg, 2010 to 2014) of heifer development systems

Item	RANGE[1]	CR[2]	DLHI[3]	DLLO[4]	SEM	*P*-value
Heifer value, $/heifer	876	876	877	877	138	1.00
Feed cost:						
Receiving diet, $/heifer	32	32	32	32	3.43	1.00
Treatment diet, $/heifer	113[a]	109[a]	152[b]	137[c]	4.87	< 0.01
Summer pasture, $/heifer	68	68	68	68	3.69	1.00
Other expenses, $/heifer	311	319	311	311	8.96	0.91
Total development cost	1,401	1,404	1,440	1,425	152	0.99
Less: cull heifer value	228[a]	127[b]	100[b,c]	69[c]	19	< 0.01
Net cost	1,173	1,277	1,340	1,356	137	0.77
Net cost per pregnant heifer, $	1,420	1,413	1,447	1,432	150	1.00

[1] RANGE = heifers were offered the equivalent of 0.45 kg · hd^{-1} · d^{-1} while grazing winter range for 170 d before entering the drylot for estrus synchronization and AI.
[2] CR = heifers were offered the equivalent of 0.45 kg · hd^{-1} · d^{-1} while grazing corn residue for 90 d and winter range for 80 d before entering the drylot for estrus synchronization and AI.
[3] DLHI = heifers were developed in the drylot for 170 d and through estrus synchronization and AI on a high-energy diet.
[4] DLLO = heifers received a low-energy diet in the drylot for 170 d through estrus synchronization and AI.
[5] Heifers received a common receiving diet for 30 d prior to the initiation of the treatments.
[6] Summer pasture was calculated as half the cost of a cow-calf pair.
[7] Other expenses included breeding expense, interest (6.5% of heifer value), yardage, trucking for heifers on CR, vaccinations and other miscellaneous health expenses.
[a,b,c] Means in a row with different superscripts are different ($P \leq 0.05$).

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Comparison of timed insemination vs. modified estrus detection protocol in beef heifers

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ABSTRACT: Angus-based, crossbred heifers (n = 972, 346 kg ± 14 kg) were assigned to either a fixed-time AI (FTAI) protocol or modified estrus detection with fixed-time AI (MTAI) to evaluate synchronization, conception, and pregnancy rates. During the pre-breeding development period, heifers were fed to achieve a target of 60 ± 5% mature BW at breeding. Heifers were synchronized via melengestrol acetate-prostaglandin $F_{2\alpha}$ (MGA-PG) protocol and received an estrus detection aid (patch) at PG administration. A patch score was recorded for each heifer at AI to reflect what percentage of rub-off coating had been removed. Heifers in the FTAI treatment received 2 mL GnRH injection and were AI 72 ± 2 h following PG. Heifers in MTAI treatment were observed for estrus at 58 ± 2 and 70 ± 2 h after PG. Approximately 72 ± 2 h after $PGF_{2\alpha}$, heifers in MTAI were AI in the following order: heifers in estrus at 58 h post-PG, heifers in estrus at 70 h post-PG, and heifers not expressing estrus at either estrus observation. Heifers not expressing estrus received GnRH at AI. Pregnancy was determined via transrectal ultrasonography. Heifers exhibiting estrus had greater ($P < 0.01$; 71 and 66 ± 5% for FTAI vs. MTAI, respectively) AI conception rates than heifers not expressing estrus in both FTAI and MTAI treatments vs. 47 and 53 ± 9 % AI conception rates in non-estrus heifers for FTAI and MTAI, respectively. However, overall AI conception rate (62 ± 5%, $P = 0.49$) and final pregnancy rates were similar ($P = 0.98$; 96 and 97 ± 3% for FTAI vs. MTAI, respectively). Similar AI conception rates were achieved without estrus detection.

Key Words: beef heifers, estrus detection, estrus synchronization, timed artificial insemination

INTRODUCTION

Using AI in replacement heifers decreases the chance for dystocia by using high accuracy calving ease sires (Bennet and Gregory, 2001). Regardless of dam age, Lamb et al. (2010) concluded synchronization of the estrous cycle can shorten the calving season, increase calf uniformity, and facilitate the use of AI. Artificial insemination allows producers to utilize superior genetics at costs less than purchasing a herd sire of similar quality.

Few beef producers have implemented AI programs, and of these producers, most produce seedstock. Estrus synchronization and AI require careful planning and additional time and labor. Consequently, adoption rates of estrus synchronization and AI have been low. Fixed-time AI (**FTAI**) protocols can minimize the number of times cattle are handled and eliminate estrus detection, but may provide lower conception rates than protocols involving estrus detection (Lamb et al. 2006). Melengestrol acetate (**MGA**) is an alternative progestin commonly used to synchronize estrus in beef heifers and has proven to be as effective as controlled internal drug release (**CIDR**) device in time AI protocols (Vraspir et al., 2013). Therefore, the objective of the present study was to compare modified estrus detection and FTAI vs. FTAI in a MGA-prostaglandin $F_{2\alpha}$ (**PG**) synchronization protocol.

MATERIALS AND METHODS

The University of Nebraska-Lincoln Animal Care and Use Committee approved the procedures and facilities used in this experiment. Yearling, Angus-based crossbred heifers (n = 972) were managed in 3 groups at the Kelley Ranch near Sutherland, NE. Initial BW (346 ± 14 kg) was similar between treatments ($P = 0.46$). During the development period, heifers were fed to achieve a target of 60% mature BW at breeding.

Heifers in Group 1 (n = 298) were managed in 3 drylot pens and offered a diet (40% DM) containing 0.6 kg/d wet distillers grains (**WDG**), 2.4 kg/d grass hay, 3.2 kg/d corn silage (**CS**), and 0.2 kg/d balancer pellet on a DM basis. Heifers in Group 2 (n = 317) grazed dormant meadow and were offered supplement (0.8 kg/d WDG, 1.4 kg/d CS, and 0.2 kg/d balancer pellet on a DM basis; 30% DM). In early February, heifers in Group 2 were moved to 2 drylot pens and offered a diet (48% DM) containing 1.0 kg/d WDG, 4.0 kg/d grass hay, 1.8 kg/d CS, and 0.2 kg/d balancer pellet on a DM basis. Heifers in Group 3 (n = 357) were managed in 5 drylot pens and offered a diet (44% DM) comprised of 0.8 kg/d WDG, 3.3 kg mixed hay (50, 25, and 25% alfalfa, grass, and millet hay, respectively), 2.7 kg CS on a DM basis, and 0.4 kg liquid finisher supplement (as-fed).

All heifers were synchronized using a MGA-PG protocol (Vraspir et al., 2013), where on d 1 to 14 each heifer was offered 0.50 mg/d MGA (Zoetis, Florham Park, NJ) pellets mixed in their diet. On d 33, heifers received a PG (Lutalyse, Zoetis, Florham Park, NJ) 5 mL i.m. injection and estrus detection aids (patches) applied (Estrotect, Rockway Inc, Spring Valley, WI). A patch score was recorded for each heifer at AI to reflect what percentage of rub-off coating had been removed. A score of 1 designated a patch with no rub-off coating removed, a score of 2 designated a patch with < 50% of the rub-off coating removed, a score of 3 designated a patch with ≥ 50% of the rub-off coating removed, and a score of 4 designated a missing patch. Heifers receiving a patch score of 3 were considered to have expressed estrus.

All FTAI heifers (Figure 1) received 2 mL GnRH (Fertagyl, Intervet/Merck Animal Health, Madison, NJ) i.m. injection and AI 72 ± 2 h following PG. Heifers in the modified-time AI (**MTAI**, Figure 2) treatment were detected for estrus at 58 ± 2 and 70 ± 2 h after PG. Heifers expressing estrus (patch score 3) were penned separately. Approximately 72 ± 2 h after PG, heifers in MTAI were AI in the following order: heifers in estrus at 58 h post-PG, heifers in estrus at 70 h post-PG, and heifers not expressing estrus at either observation time. Heifers not expressing estrus received GnRH at AI. Thirteen days following AI, bulls were placed with heifers at a bull to heifer ratio of 1:50 for a 42 d breeding season. A minimum of 51 d after AI, BW was measured and pregnancy was detected via transrectal ultrasonography (Aloka, Hitachi Aloka Medical America Inc., Wallingford, CT). Heifers not pregnant by AI were weighed and diagnosed for pregnancy 45 d following bull removal.

Statistical Analysis

All data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, N.C.) accounting for group, location, treatment, and treatment x location interaction. Group, location, and AI technician were included as random variables. Pregnancy rate was analyzed using an odds ratio. Least squared means and SE of the proportion of pregnant heifers by treatment were obtained using the ILINK function.

RESULTS AND DISCUSSION

Breeding Treatment

Heifer reproductive performance is presented in Table 1. Pre-breeding BW was similar (P = 0.48) between FTAI and MTAI treatment groups (345 and 348 ± 14 kg, respectively). Furthermore, BW was similar (P = 0.26) at first pregnancy diagnosis (366 and 369 ± 7 kg; FTAI and MTAI, respectively). Heifers from both groups reached a similar (P = 0.86) percentage mature BW (62 ± 5%, based on 553 kg mature BW) prior to breeding. At the second pregnancy diagnosis, BW (P = 0.05) was 411 and 417 ± 8 kg for FTAI and MTAI, respectively.

The AI conception rate was similar (62 ± 5%, P = 0.49) for both treatments. Conception rates by patch score in FTAI were 42, 48, 71, and 41 ± 5% for patch scores 1 (n =44), 2 (n = 144), 3 (n = 283), and 4 (n = 15), respectively. Conception rates by patch score in MTAI were 52, 53, 66, and 55 ± 5% for patch scores 1, 2, 3, and 4. Heifers exhibiting an activated patch (score 3) had greater (P < 0.01; 71 and 66 ± 5% for FTAI and MTAI, respectively) AI conception rate in both FTAI and MTAI treatments vs. 47 and 53 ± 9 % AI conception rates in non-estrus heifers (score 1, 2, and 4) for FTAI and MTAI, respectively. At first estrus detection (58 h) 132 heifers exhibited a patch score of 3 (66 ± 5% conception rate), at second estrus detection (70 h) 156 heifers exhibited a patch score 3 (66 ± 5% conception rate), and at AI 38 additional heifers exhibited a patch score 3 for MTAI protocol (68 ± 5% conception rate). Estrus activity at AI did not influence final pregnancy rates (96 and 97 ± 3% for FTAI vs. MTAI, respectively; P = 0.97).

Echternkamp and Thallman, (2011) found cows expressing estrus prior to TAI had greater pregnancy rates than cows not expressing estrus. This is supported by Perry et al., (2005) who suggests females expressing estrus prior to TAI have greater pregnancy rates than non-estrus animals.

Mallory et al., (2010) compared MGA and CIDR synchronization protocols, finding no difference in AI pregnancy rate. However, interval to estrus after PG injection tended to be greater in heifers synchronized with a CIDR, which suggests the MGA-PG protocol may have an advantage over CIDR protocol in producing a tighter synchrony among heifers (as reviewed by Nielson et al., 2015). Additionally, Vraspir et al. (2013) observed similar FTAI pregnancy rates in heifers synchronized with MGA vs. 14-d CIDR. In the present study, the MGA-PG protocol synchronized the estrus cycle in FTAI treatment to allow heifers to attain estrus at or near FTAI, which is supported by the similar AI conception rate (62 ± 5%) observed for both FTAI and MTAI, suggesting proper alignment of ovulation and AI.

In a previous study on the same ranch, Nielson et al. (2015) evaluated a 19-h delayed AI following GnRH injection in a hybrid estrus detection and FTAI protocol. Heifers were synchronized, detected for estrus, and AI similar to the present study. Seventy-two h following PG, heifers not detected in estrus were administered GnRH, and randomly assigned to 1 of 2 treatment groups: 1) immediately AI or 2) AI 19 ± 1 h later. Heifers in estrus prior to AI had a greater pregnancy rate than those time AI. Delaying the time of AI did not increase pregnancy rates (70, 56, and 47 ± 6%; heifers in estrus, AI 72 h, AI 72 + 19 h later, respectively), concluding the additional labor of delaying AI was not justified.

The present study evaluated a modified estrus detection and FTAI protocol vs. a FTAI with no estrus detection, and similar to Nielson et al. (2015) there was no advantage in AI conception rates, negating the need for additional labor. Data from the present study suggests similar conception rates may be achieved by FTAI when compared with a protocol involving estrus detection.

IMPLICATIONS

Assisted reproductive technologies such as estrus synchronization and AI have limited adoption in the beef industry, partially due to added complexity, labor, and potential perceived reproductive risk. Protocols that limit labor and cattle processing have a greater potential of being adopted. The present study provided a synchronization and AI protocol that limits cattle handling and eliminates estrus detection without compromising conception rates compared with a more labor intensive protocol utilizing estrus detection.

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Table 1. Reproductive performance of heifers on a FTAI[1] or MTAI[2] synchronization protocol.

Item	FTAI				MTAI				SEM	*P*-Value
Pre-breeding BW, kg	346				344				14	0.87
Pregnancy test BW, kg	366				369				7	0.27
2nd Pregnancy test BW[3], kg	411				417				8	0.05
ADG[4], kg	0.4				0.3				0.05	0.59
Percent Mature BW[5], %	62				63				5	0.86
AI Pregnancy Rate, %	62				62				5	0.49
Final Pregnancy Rate, %	96				97				3	0.98
Patch Score[6]	1	2	3	4	1	2	3	4		
AI Pregnancy Rate[7], %	42[b]	48[b]	71[a]	40[b]	52[b]	53[b]	66[a]	55[b]	8	< 0.05
Final Pregnancy Rate, %	96	96	97	86	93	90	95	99	3	0.97

[1] FTAI = synchronized using melengestrol acetate-prostaglandin $F_{2\alpha}$ (MGA-PG) protocol, d 1 to 14 heifers offered 0.50 mg/(hd ·d) MGA (Zoetis, Florham Park, NJ),d 33, PG (Lutalyse, Zoetis, Florham Park, NJ) 5 mL i.m. injection and estrus detection aids (Estrotect, Rockway Inc, Spring Valley, WI). Approximately 72 ± 2 h after PG heifers received GnRH and AI.
[2] MTAI = synchronized using MGA-PG protocol. Approximately 72 ± 2 h after PG, heifers were AI in the following order: heifers in estrus 58 h post-PG, heifers in estrus 70 h post-PG, and heifers not expressing estrus given GnRH.
[3] Second pregnancy diagnosis BW occurred at a minimum 50 d following first pregnancy diagnosis
[4] ADG from pre-breeding to pregnancy diagnosis (57 d).
[5] Based on 553 kg mature BW.
[6] Patch score 1 = not rubbed; 2 = ≤ 50% rubbed; 3 = ≥ 50% rubbed; 4 = missing estrus detection patch.
[7] Means [a,b] in a row with differing superscripts are different ($P < 0.05$)

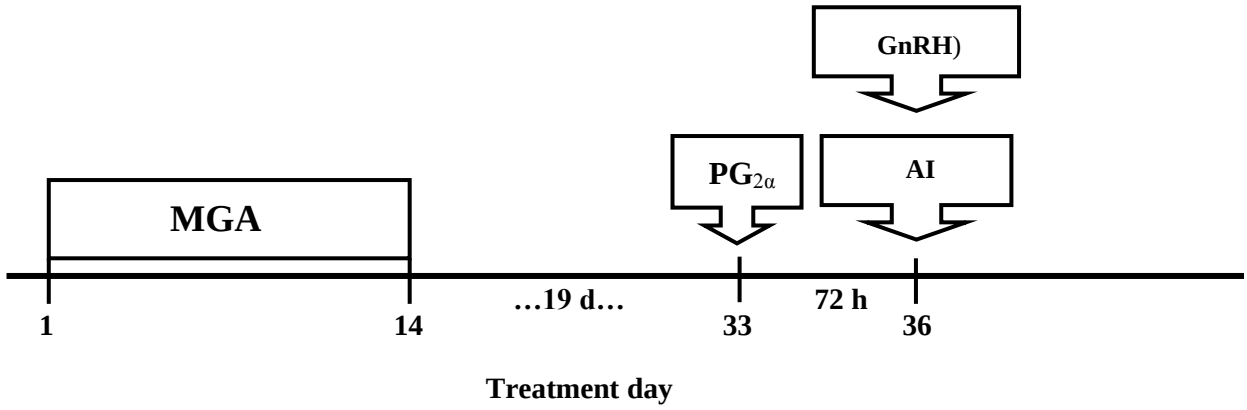

Figure 1. Fixed-time AI melengestrol acetate (MGA) - $PGF_{2\alpha}$ synchronization protocol

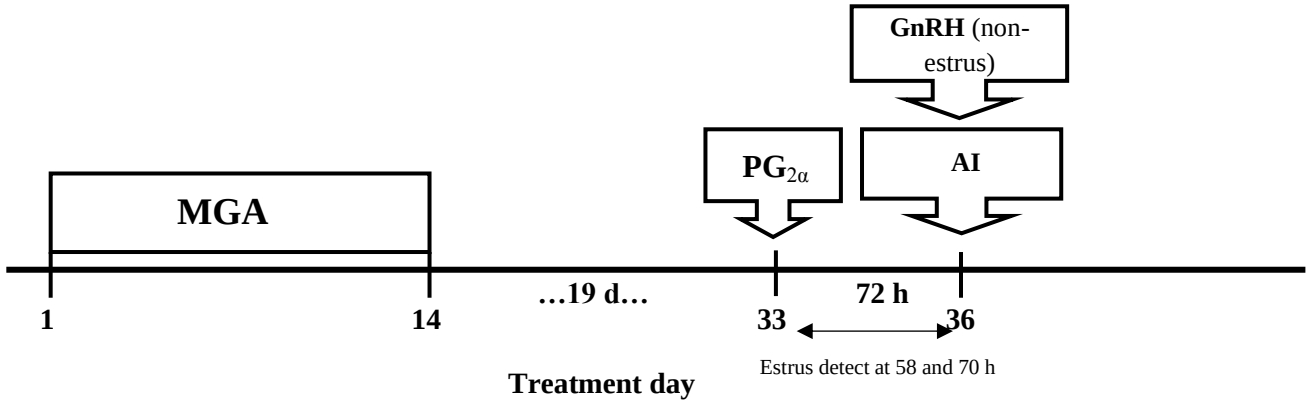

Figure 2. Modified melengestrol acetate (MGA) - $PGF_{2\alpha}$ synchronization protocol

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Growth and reproductive performance of yearling beef heifers implanted with Revalor G in the Nebraska Sandhills

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ABSTRACT: Crossbred beef heifers (n = 3,242), approximately 12 mo of age, were managed at 3 locations in the Nebraska Sandhills and randomly assigned to be implanted with Revalor G (40 mg of trenbolone acetate and 8 mg estradiol, IMP), while the control group (CON) did not receive an implant. Heifers (238 ± 2 kg) grazed native Sandhills range for the duration of the trial (164 ± 4 d). Eighty-two ± 2 d following trial initiation, heifers were synchronized for estrus and AI followed with clean-up bulls as part of a 25 d breeding season. Body weight was measured at the beginning and end of trial. Pregnancy detection occurred 45 d following bull removal at the conclusion of the summer grazing period. Implanted heifers gained more and were heavier ($P < 0.05$; 0.68 vs. 0.64 ± 0.01 kg/d and 347 vs. 340 ± 3 kg, IMP vs. CON, respectively) at the end of the trial. However, pregnancy rate was greater ($P < 0.01$) for CON vs. IMP (64 vs. 46 ± 3% respectively). Implanted heifers also had a lower pregnancy rate in their second breeding season ($P = 0.02$; 93 vs. 96 ± 2%, IMP vs. CON, respectively). Implanting beef heifers with Revalor G at approximately 12 mo of age increased ADG and summer BW gain; however, it decreased initial and subsequent pregnancy rate compared with heifers not implanted.

Key words: beef heifers, fertility, growth implants

INTRODUCTION

Administering growth implants in stocker systems results in increased growth, improved efficiency, and increased profitability (Barham, 2003). Initially, growth implants were utilized in the finishing phase of production, but over the past several decades, growth implants have been incorporated at earlier stages of growth and development. Anabolic implants increase stocker cattle BW gains by 8 to 18% or 7 to 18 kg during the grazing season (Kuhl, 1997; Selk, 1997). Kuhl (1997) reported data from 3 studies (n = 494), in which stocker heifers receiving a Revalor G implant gained 12 kg more than non-implanted controls, which was 4.6% greater than responses to Ralgro during a 116-d grazing period. However, growth implants have not been widely used in heifer calves, due to subsequent reproductive concerns (Selk, 1997). Consequently, less research has been conducted with heifers. Reproductive performance has been variable; however, several studies have shown decreased reproductive performance of beef heifers implanted once with Ralgro at weaning (Nelson et al., 1972; Pruitt et al., 1980; Pritchard et al., 1989). Traditional heifer development programs focus on maximizing reproductive rates; therefore, reproductive risk associated with implants has been avoided. However, if excess beef females are retained after weaning, a management strategy may be to implant heifers and accept a decreased conception rate; but increase stocker gains in non-pregnant heifers, provided an adequate number of replacements are achieved.

Increased growth responses to implants are consistent, but reproductive performance in beef heifers has been variable. Therefore, objectives of the present study were to evaluate effects of a single stocker implant (Revalor G) on growth and reproductive performance of yearling beef heifers in the Nebraska Sandhills.

MATERIALS AND METHODS

In 2011, 12 mo old crossbred beef heifers grazing native Sandhills range at 3 locations were randomly assigned to be implanted with Revalor G (Merck Animal Health, Summit, NJ; 40 mg trenbolone acetate and 8 mg estradiol; **IMP**) or not implanted (control, **CON**). Heifers were implanted at the beginning of the grazing period (May 1). Initial heifer BW was similar ($P = 0.03$) between treatments (238 ± 2 kg). At the time of implant, all heifers were vaccinated (Pyramid 5, Boehringer Ingelheim, St. Joseph, MO; and VL5 Staybred, Zoetis, Florham Park, NJ) and treated with a topical endectocide (Ivermax, RXV Products, Westlake, TX). At each location, heifers grazed common upland pastures for 164 ± 4 d.

A 25 d breeding season began 82 ± 2 d following trial initiation. Heifers at location 1 (**L1**, n = 942) were synchronized with 2 prostaglandin $F_{2\alpha}$ (**PG**) injections administered 17 d apart (5 ml, Lutalyse, Zoetis, Florham Park, NJ) followed by 5 d of estrus detection and AI. Mature bulls were then placed with heifers at a 1:52 bull to heifer ratio for 20 d to conclude a 25 d breeding season. At location 2 (**L2**; n = 1,184) and 3 (**L3**; n = 1,116), mature bulls were placed with heifers at a 1:82 bull to heifer ratio 6 d before heifers received a single PG injection followed by 6 d of estrus detection and AI. Estrus detection aids were utilized at all 3 locations (Estrotect, Rockway Inc., Spring Valley, WI) at PG injection. Heifers were considered to have expressed estrus when greater than 50% of the rub-off coating had been removed from the Estrotect patch and were AI 12 h later. Following the AI period, mature bulls were then placed with heifers at ratios of 1:49 and 1:35 at L2 and L3, respectively, for 19 d to conclude a 25 d breeding season.

Heifers were managed on native Sandhills range throughout the summer grazing period. Pregnancy diagnosis was conducted via transrectal palpation approximately 45 d following bull removal and ending BW measured. Non-pregnant heifers were marketed as

stocker cattle. During the second production year, heifers (n = 1,667; 706 and 961, IMP and CON, respectively) retained as replacements were managed in 3 groups and grazed native upland range throughout the year without further treatment. Cows were offered 0.45 kg/d of a 32% CP supplement range cube for 30 days (15 d prior to breeding until 15 d following bull turnout (July 25). Pregnancy diagnosis was performed via transrectal palpation approximately 45 d following bull removal.

Economic Evaluation

Heifer development economic analysis was performed similar to Summers et al. (2014), and is presented in Table. 2. Winter grazing cost was estimated to be one-half the grazing costs for a mature cow ($0.46/d) based on heifer BW at weaning, as previously established (Larsen et al., 2011). Winter range with supplement was valued at $0.75/d. Summer grazing costs, $0.55/d for upland grass, were based on Johnson et al., (2010). Additional development costs, including feed delivery costs, breeding costs, and health and veterinarian costs, were charged at $0.36/d. Average heifer purchase and cull prices were based on USDA Agricultural Marketing Service prices reported in Nebraska for each date (USDA-AMS, 2008). Net cost of 1 pregnant heifer was calculated using the formula developed by Feuz (1992). The total value of cull heifers was subtracted from the total cost of all developed heifers. Total costs were then divided by the number of heifers exposed to determine the total cost of 1 pregnant heifer. This value was divided by final pregnancy rate to determine the total net cost of 1 pregnant heifer.

Statistical Analysis

Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, N. C.). Individual heifer was the experimental unit and synchronization protocol was included as a random variable in the model. Location was experimental unit for economic analysis and in Table 2 where data are presented by location. Least squares mean and SE for ADG, BW, and pregnancy rate were obtained using the Tukey function of SAS.

RESULTS AND DISCUSSION

Heifer growth and reproductive performance are presented in Table 1 and presented by location in Table 2. Implanted heifers had greater ADG and ending BW ($P < 0.05$; 0.68 vs. 0.64 ± 0.01 kg/d and 347 vs. 340 ± 3 kg for IMP and CON, respectively). Summer gains were greater ($P = 0.03$) for IMP (110 ± 3 kg) vs. CON (104 ± 3 kg). Kuhl (1997) reported response to growth implants to be 7 to 18 kg during the summer grazing period for stocker cattle. Implanted heifers gained an average of 6 kg more than CON heifers, which is slightly lower than reported by Kuhl (1997). Heifers in the current study grazed native upland Sandhills pasture during the trial without supplement. Forage quality of Sandhills rangeland early in the grazing period is high, but decreases with increasing plant maturity (Lamb, 1996). Therefore, heifers on a higher plane of nutrition for the entire grazing period would likely have a greater growth response to implants. Additionally, a synergistic growth response for implanting in combination with supplementation is commonly observed in stocker cattle, where nutrient deficiencies are corrected, or forage resources extended, via supplementation strategies (Kuhl, 1997). In a Missouri study, providing late-season supplementation to stocker calves improved ADG in re-implanted yearlings (Sewell, 1983).

In a 2-yr Montana study, weaned heifers were separated into 2 weight classes and divided between implant and non-implanted control. Heifers were developed in a drylot until 1 mo prior to breeding season. Weaned heifers were implanted with Zeranol at 8 and 11 mo of age. Rates of gain in the drylot were greater for implanted vs. control heifers in both Trial 1 (0.53 vs. 0.48 kg/d) and Trial 2 (0.70 vs. 0.63 kg/d). Pregnancy rate was 16 percentage points lower in implanted heifers vs. control (62 vs. 78%) in Trial 1, but did not differ (88 vs. 87%; implant vs. control, respectively) in Trial 2 (Staigmiller et al., 1983).

In a similar study, implants were administered to crossbred beef heifers at 1, 6, or 9 mo, or at multiple intervals. Heifers receiving a combination of 2 implants had greater ADG from weaning to breeding than control or heifers implanted 3 times. Conception rates in a 62-d breeding season were comparable for implanted vs. non-implanted control heifers (93 vs. 96%), with the exception of heifers receiving implants at both 1 and 6 mo of age (56%). Calf birth weight, dystocia score, cow re-breeding rate, and calf weaning weight were not affected by implant treatment (Deutscher et al., 1984).

In the present study, pregnancy rate was greater ($P < 0.01$) for CON vs. IMP heifers (64 vs. 46 ± 3%). This is consistent with results from Trial 1 in Staigmiller et al. (1983), which demonstrated a 16 percentage point reduction in pregnancy rate in implanted heifers. However, our data contrasts Staigmiller et al. (1983) Trial 2, which resulted in similar pregnancy rates regardless of implant treatment. In the Trial 2, heifers were fed to reach greater pre-breeding BW (293 kg vs. 341 kg, Trial 1 vs. Trial 2, respectively), which may explain differences in pregnancy rates. In the present study, heifers were developed to a similar pre-breeding BW (294 vs. 290 kg in IMP and CON, respectively) as Trial 1. Additionally, age at implant may also explain differences observed between previous research and the present study. Staigmiller et al., (1983) implanted heifers at 8 and 11 mo of age; in the present study, heifers were implanted at 12 mo of age. Additionally, Deutscher et al. (1984) reported similar conception rates among non-implanted controls and heifers implanted at 1, 6, or 9 mo of age, earlier than the present study. In addition to age, type of implant may also contribute to variation in pregnancy rates. Ralgro was utilized in both Staigmiller et al. (1983) and Deutscher et al. (1984), Revalor G was used in the present study. Subsequent pregnancy rate after the first calving season was also lower ($P = 0.02$) in IMP (93%) vs. CON (96%) heifers, which suggests implanting heifers may have a

residual or development effect on growing heifers beyond the production yr the implant was administered.

Economic Analysis

The economic analysis is presented in Table 3. Heifers were developed together by location; therefore, winter and summer feed costs and total development costs were similar between treatments (P = 1.0). Additionally, the net cost of 1 pregnant heifer tended (P = 0.13) to be greater in CON heifers. Cull value did not differ (P = 0.66) despite a $21 numerical advantage for IMP heifers.

Stocker enterprises commonly market cattle in late summer when pasture availability or forage quality may be declining. A disadvantage to the present study is the expense and resource allocation associated with retaining heifers until pregnancy determination. It's likely that heifers continued to gain during the extended period prior to pregnancy detection; however, the increased gain due to implant had presumably diminished due to implant potency and declining forage quality.

In recent years, the beef industry has seen a decline in cattle numbers and high demand for replacement females. Some beef stocker enterprises have utilized their resources to market pregnant replacement females. Many cow-calf producers have retained all heifers for breeding and marketed excess pregnant females in response to market demand. When pregnant heifer value exceeds feeder heifer value, it is unlikely the additional BW gain in cull females will compensate for the decreased pregnancy rate. However, when pregnant heifer value is comparable to feeder heifer value, the additional BW gain from the implant increases the value and efficiency of stocker heifers.

IMPLICATIONS

Implanting beef heifers at approximately 12 mo of age with Revalor G increased heifer ADG and BW; however, implant also decreased pregnancy rate by approximately 18 percentage points. When deciding to implant replacement females, the current (or expected) market conditions for pregnant heifers and feeder heifers must be considered.

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Table 1. Effects of Revalor-G on reproduction and summer BW gain of beef heifers grazing native Sandhills rangeland

Item	CON[1]	IMP[2]	SEM	*P*-value
n	1,621	1,621		
Spring BW, kg	237	238	2	< 0.01
Fall BW, kg	340[b]	347[a]	3	< 0.01
Summer gain, kg	104[b]	110[a]	3	< 0.01
ADG[3], kg	0.63[b]	0.67[a]	0.01	< 0.01
Pregnancy rate, %	64[a]	46[b]	3	< 0.01
2nd preg. rate, [4] %	96[a]	93[b]	2	0.02

[1]CON = Heifers did not receive a growth implant prior to breeding season.
[2] IMP = Heifers received a Revalor G implant 82 ± 2 d prior to breeding season (Merck Animal Health, Summit, NJ).
[3] Grazing season ADG (Location 1-162 d, Location 2-160 d, Location 3-168 d).
[4]Second season pregnancy rates (n = 1,667).
[a,b] Means in a row with different superscripts differ ($P < 0.05$).

Table 2. Effects of Revalor G on reproduction and summer BW gain of beef heifers grazing native Sandhills rangeland by location

Item	CON[1]			IMP[2]			SEM	*P*-value
Location	L1	L2	L3	L1	L2	L3		
Spring BW, kg	232	235	245	232	236	247	4	0.20
Fall BW, kg	326	351	359	332	360	365	15	0.02
Summer gain, kg	94	116	113	99	123	118	10	0.03
ADG, kg[3]	0.58	0.72	0.67	0.61	0.77	0.70	0.06	0.03
Pregnancy rate, %	59	64	67	44	44	51	3	< 0.01

[1]CON = Heifers did not receive a growth implant prior to breeding season.
[2]IMP = Heifers received a Revalor G (Merck Animal Health, Summit, NJ) implant 82 ± 2 d prior to breeding season.
[3]Grazing season ADG (Location 1, 162 d; Location 2,160 d; Location 3, 168 d).

Table 3. Economics of implanting beef heifers with Revalor G at 12 mo of age[1]

Item	CON[2]	IMP[3]	SEM	*P*-value
Winter feed costs /$heifer[4]	102	102	.02	1.0
Summer feed cost /$heifer	91	91	.1	1.0
Total feed costs, $/heifer	193	193	.02	1.0
Total development cost[5] $/heifer	1,019	1,019	3	1.0
Avg. cull heifer value $	1,102	1,123	46	0.66
Cull heifer value $/heifer exposed	402	601	18	< 0.01
Net cost of 1 pregnant heifer[6], $	969	901	36	0.13

[1]Heifers developed at Rex Ranch on native Sandhills rangeland.
[2]CON = Heifers did not receive a growth implant prior to breeding season.
[3]IMP = Heifers received a Revalor G (Merck Animal Health, Summit, NJ) implant 82 ± 2 d prior to breeding season.
[4]Heifers grazed winter range for 135 d with the equivalent of 0.45 kg/d 32% CP supplement 3 times per wk.
[5]Includes all fixed and variable cost associated with initial heifer price, feed, feed delivery, breeding, transportation, and supplement.
[6]Total value of cull heifers was subtracted from the total cost of all developed heifers. Total costs were then divided by the number of heifers exposed to determine the total cost of 1 pregnant heifer.

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Bioavailability of supplemental ruminally-protected leucine in sheep[1]

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ABSTRACT: The objective of this study was to evaluate the effects of rumen-protected L-Leu on plasma branched-chain AA concentrations and rumen fermentation characteristics of lambs. Four ruminally-cannulated wether lambs (34 ± 2.4 kg BW) were used in a 4 × 4 Latin square. Each period consisted of 7 d, 5 d for adaptation, 1 d for collections, and 1 d of rest. Lambs were fed a basal diet (corn grain and alfalfa hay; 0.6 kg/d DM) and supplements (0.1 kg/d DM) containing no added Leu (**CON**), 6 g/d of unprotected L-Leu (**UP-LEU**), and 18 g/d ruminally-protected L-Leu (**RP-LEU**), or post-ruminally infused with 6 g/d of L-Leu (**INF-LEU**). Blood and rumen fluid samples were collected on d 6 of each period at 0, 3, 6, and 9 h after feeding. The statistical model included period, sheep, treatment, h, and treatment × hour. Lambs receiving INF-LEU had plasma Leu concentrations that were greater at 3 and 6 h, but not different at 9 h compared to CON, UP-LEU, and RP-LEU (treatment × h; $P < 0.01$). Plasma Ile concentrations were lower for RP-LEU than CON, UP-LEU, and INF-LEU at 0 h, lower for INF-LEU than CON, UP-LEU, RP-LEU and at 3 h, not different among treatments at 6 h, and lower for RP-LEU and INF-LEU than CON and UP-LEU at 9 h (treatment × h, $P = 0.02$). Rumen fluid acetate (mol/100 mol) tended to be lower for RP-LEU than CON, UP-LEU, and INF-LEU at 0 h, lower for UP-LEU than CON, RP-LEU, and INF-LEU at 3 h, not different among treatments at 6 h, and greater for UP-LEU than CON, RP-LEU, but not INF-LEU, at 9 h (treatment × h, $P < 0.01$). Rumen isovalerate (mol/100 mol) was greatest for RP-LEU, intermediate for UP-LEU, and lowest for INF-LEU and CON ($P < 0.01$). Rumen fluid pH, NH_3, total VFA, and molar proportions of propionate, isobutyrate, butyrate, valerate, and acetate:propionate ratio were not altered by treatments ($P \geq 0.01$). Although supplementation of RP-LEU was unable to elevate plasma Leu concentrations, decreases in plasma Ile concentrations are likely due to the antagonistic effects of post-absorptive L-Leu on plasma Ile concentrations. This data implies that the ruminally-protected Leu was absorbed by the gastrointestinal tract of lambs. Altered rumen fermentation also demonstrated that the ruminally-protected L-Leu source was not entirely protected from rumen microorganisms.

Key words: leucine, rumen-protected, sheep

INTRODUCTION

Greenwood and Titgemeyer (2000) demonstrated that Met, His, Lys, and at least one branched-chain AA (**BCAA**) were limiting in growing steers. Löest et al. (2001) demonstrated that Leu and Val were limiting BCAA when growing steers were fed a diet low in RUP. Nolte et al. (2008) demonstrated that Met, Thr, Arg, Trp, and Val were limiting in growing lambs, and concluded that supplementation of these limiting AA in a rumen-protected form could improve performance. Effects of BCAA on milk protein synthesis by dairy cows have also been studied (Mackle et al., 1999; Appuhamy et al., 2011).

Branched-chain AA, particularly Leu, serve an imperative role in the regulation of free AA and protein metabolism. These metabolic functions include effects on pancreatic secretion of insulin, regulation of protein synthesis in certain cells, and interorgan signaling pathways (Kuhara et al., 1991; Papet et al., 1992). In a review, Calder (2006) reported that the immune system is dependent on BCAA for synthesis of acute-phase response proteins. Waggoner et al. (2009) demonstrated an inverse relationship between nitrogen excretion and plasma concentration of BCAA in steers exposed to lipopolysaccharide (LPS), and Gilliam et al. (2008) exhibited that calves exposed to LPS had less nitrogen loss when post-ruminally infused with BCAA. Therefore, BCAA appear to decrease catabolic protein loss in stressed calves. Carter et al. (2011) reported an increase of bovine ovalbumin specific immunoglobulin when rumen-protected BCAA were supplemented to beef cattle.

Due to supporting evidence that supplemental BCAA enhance protein synthesis for growth performance, milk production, and immune cells of cattle and sheep, development of rumen-protected BCAA for supplementation in diets of ruminants may be beneficial to the livestock industry. The objective of this research was to evaluate the effects of a rumen-protected Leu product on plasma BCAA concentrations and rumen fermentation characteristics of lambs.

MATERIALS AND METHODS

Animals, Facilities, and Diet.

All procedures for this study were approved by the Institutional Animal Care and Use Committee at New Mexico State University. Four ruminally-cannulated wether lambs (34 ± 2.4 kg initial BW) were housed individually in adjacent soil-surfaced pens (3 × 3 m) with shades and free access to water. Lambs were fed (0.6 kg DM/d) twice daily

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a basal diet (Table 1) in equal proportions at 0700 and 1900.

Experimental Design and Treatments

The experiment was a 4 × 4 Latin square, with lamb as the experimental unit. Each period was 7 d, allowing 5 d for adaptation to treatments, 1 d for collections, and 1 d of rest. Treatments (Table 2) were supplements (fed at 100 g/d DM) containing no added Leu (**CON**), 6 g/d of an unprotected source of L-Leu (**UP-LEU**)**,** and 18 g/d of a ruminally-protected source of L-Leu (**RP-LEU**; contained 45% L-Leu and 75% ruminally-protected). The fourth treatment was a positive control, where lambs received the CON supplement and were post-ruminally infused with an L-Leu solution (**INF-LEU**). The L-Leu solution (2.5% w/v) was prepared with deionized water and stored at 4°C before every 7 d period. Treatments were fed or infused (240 mL/d) twice daily at 0700 and 1900. Flexible tubing was placed through the rumen cannula and reticulo-omasal orifice, and anchored in the abomasum with a rubber flange (3 cm in diam.) which allowed for post-ruminal infusions as described by Löest et al. (2001). Lambs not receiving INF-LEU were abomasally infused twice daily with water (240 mL/d).

The basal diet plus dietary supplements were formulated to supply MP (67.2 g/d) and metabolizable BCAA (5.9, 3.4, 3.9 g/d for Leu, Ile, and Val, respectively) in excess of requirements (48.3, 3.3, 1.5, and 1.7 g/d for MP, Leu, Ile, and Val, respectively), which were calculated using the net protein requirement (equations of NRC, 1985) for 0.26 Mcal/d NEg and the whole body essential AA composition of lambs (NRC, 2007). Metabolizable AA supply from microbial CP was calculated based on microbial protein synthesis (13% of TDN intake; NRC, 2000), the AA composition of microbial protein (Storm and Ørskov, 1983), and biological value of 0.64 (NRC, 2000). Metabolizable AA supply from RUP was calculated based on the weighted average of the AA composition of RUP for each feed ingredient in the diet (NRC, 2000).

Collections

On d 6 of each period, blood samples were collected via jugular venipuncture at 0, 3, 6, and 9 h after the 0700 feeding and supplement delivery. These collection times were selected because Bach and Stern (2000) demonstrated that plasma Met concentrations peaked between 6 and 12 h after dosing a medium-slow degradable ruminally-protected Met. Blood was collected into vacuum tubes with sodium heparin (Monoject, Mansfield, MA), immediately chilled on ice, and then centrifuged (5804, Eppendorf, Newport Beach, CA) for 20 min at 2,000 × *g*. Plasma was transferred into polypropylene vials and stored at -20°C until analysis. Rumen fluid samples were also collected on d 6 at 0, 3, 6, and 9 h after feeding. Rumen fluid was strained through 4 layers of cheesecloth and the pH was recorded using a portable pH meter (Mettler Toledo, Schwerzenbach, Switzerland). Aliquots of rumen fluid were transferred into 2 vials (20-mL) and stored at -20°C for later analysis.

Sample Analysis

Plasma was prepared using a commercially available AA kit (E:Z Faast KGO-7165; Phenomenex, Torrance, CA) and concentrations of Leu and Ile were analyzed by gas chromatography (Agilent 7890A; Agilent technologies, Santa Clara, CA). Frozen rumen fluid samples were allowed to thaw and then centrifuged (5415 R Eppendorf North America) at 16,000 × *g* for 10 min. The supernatant was separated and 200 µL of a 25% (w/v) metaphosphoric acid and 0.216% (v/v) 2-ethylbutyrate were added. Gas chromatography (Agilent 7890A) analysis was completed to determine VFA concentrations in rumen fluid according to the methods of May and Galyean (1996). Analysis of NH_3 concentrations in rumen fluid were conducted using colorimetric techniques as described by Broderick and Kang (1980).

Statistical Analysis

Data were analyzed as a 4 × 4 Latin Square using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with repeated measures (covariant structure was compound symmetry) within period. The statistical model included period, sheep, treatment, h, and treatment × h, with sheep(period×treatment) as random. Least squares means were used to present data, and differences with $P \leq 0.05$ were considered significant. Differences were considered tendencies when $0.05 < P < 0.10$.

RESULTS

Plasma Branched-Chain AA

Plasma Leu concentrations (Fig. 1) of lambs receiving INF-LEU increased from 0 h and were greater at 3 (peak) and 6 h, but not different at 9 h when compared to CON, UP-LEU, and RP-LEU; plasma Leu was not different among UP-LEU, RP-LEU, and CON (treatment × h; $P < 0.01$). Plasma Ile concentrations (Fig. 1) were lower for RP-LEU than CON, UP-LEU, and INF-LEU at 0 h, lower for INF-LEU than CON, UP-LEU, RP-LEU and at 3 h, not different among treatments at 6 h, and lower for RP-LEU and INF-LEU than CON and UP-LEU at 9 h (treatment × h, $P = 0.02$). Because of missing Val values associated with unidentifiable peaks on the chromatograph, results for plasma Val concentrations were not reported.

Rumen Fermentation

Molar percentages (mol/100 mol) of acetate (Fig. 2) tended to be lower for RP-LEU than CON, UP-LEU, and INF-LEU at 0 h, lower for UP-LEU than CON, RP-LEU, and INF-LEU at 3 h, not different among treatments at 6 h, and greater for UP-LEU than CON, RP-LEU, but not INF-LEU, at 9 h (treatment × h, $P < 0.01$). Molar percentages of isovalerate (Table 3) were greatest for RP-LEU, intermediate for UP-LEU, and lowest for INF-LEU and CON ($P < 0.01$). Rumen fluid pH, NH_3, total VFA, and molar proportions of propionate, isobutyrate, butyrate,

valerate, and acetate:propionate ratio were not altered ($P \geq 0.01$) by treatments (Table 3).

Table 1. Composition of the basal diet

Item	% of DM
Ingredient, %	
Corn grain, cracked	50.7
Hay, alfalfa	40.0
Molasses	5.0
Cottonseed meal	3.0
Urea	0.50
Mineral premix[1]	0.50
Salt	0.30
Nutrient analysis[2]	
NDF	22.0
ADF	15.1
CP	14.4
K	1.43
Ca	0.72
P	0.32
S	0.27

[1]Contained 14 to 16.5% Ca; ≥ 11% P; 11 to 13.5% salt; ≥ 0.50% Mg; ≥ 0.10% K; ≥ 15 mg/kg Se; ≥ 1980 mg/kg Zn; ≥ 660 KIU/kg vitamin A; ≥ 165 KIU/kg vitamin D; ≥ 1.32 KIU/kg vitamin E (Oñate Feed Co., Albuquerque, NM).
[2]Analyzed by a commercial laboratory (SDK Laboratories, Hutchinson, KS).

Table 2. Composition of supplements

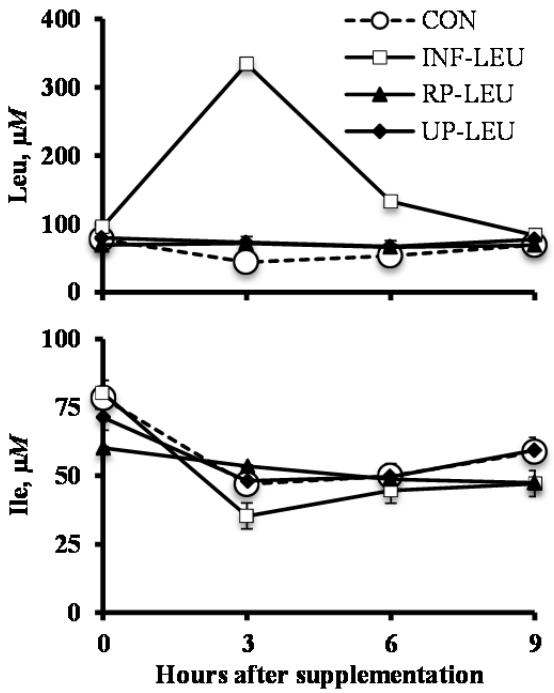
	Treatments[1]			
Item, % of DM	CON	UP-LEU	RP-LEU	INF-LEU
Corn grain, cracked	40.0	34.0	30.0	40.0
Alfalfa hay, ground	40.0	40.0	40.0	40.0
Molasses	12.0	12.0	12.0	12.0
Control prill[2]	8.0	8.0	0.0	8.0
Rumen-protected L-Leu prill[3]	0	0	18	0
Pure L-Leu[4]	0	6	0	0

[1]The INF-LEU was similar to CON, except that lambs assigned to INF-LEU were abomasally infused with a solution to supply 6 g/d of L-Leu.
[2]Similar to rumen-protected L-LEU prill but contained no L-Leu.
[3]Developed at New Mexico State University.
[4]Product of Anjinomoto, (Limeira, Brasil).

DISCUSSION

Our hypothesis was that post-ruminal infusion of Leu, and supplementation of ruminally-protected Leu, would increase plasma concentrations in sheep. According to Merchen and Titgemeyer (1992), plasma concentrations of an essential AA will not increase until the supply of that AA exceeds requirements. Therefore, the composition of the basal diet and supplements used in this experiment were formulated to exceed the lamb's BCAA requirements. Observed increases in plasma Leu concentrations for INF-LEU, but not RP-LEU, when compared to CON could be interpreted that the rumen-protected L-Leu source was not protected from microbial degradation in the rumen. However, lower plasma Ile concentrations for both RP-LEU and INF-LEU compared to CON could indicate that the ruminally-protected L-Leu source was protected from microbial degradation and was absorbed from the gastrointestinal tract. Plasma Ile responses to RP-LEU and INF-LEU likely occurred because of BCAA antagonism (Harper et al., 1984). According to Harper et al. (1984), the α-ketoacid of Leu (α-ketoisocaporate) increases the activity of branched-chain α-ketoacid dehydrogenase, which increases the oxidation of BCAA for metabolic fuels, thus decreasing blood circulation of Val and Ile. The plasma Ile responses to supplemental rumen-protected and abomasally infused L-Leu in the current study is supported by increases in plasma concentrations of Ile and Val when Leu was removed from essential AA infusions in cattle (Löest et al., 2001) and sheep (Nolte et al., 2008).

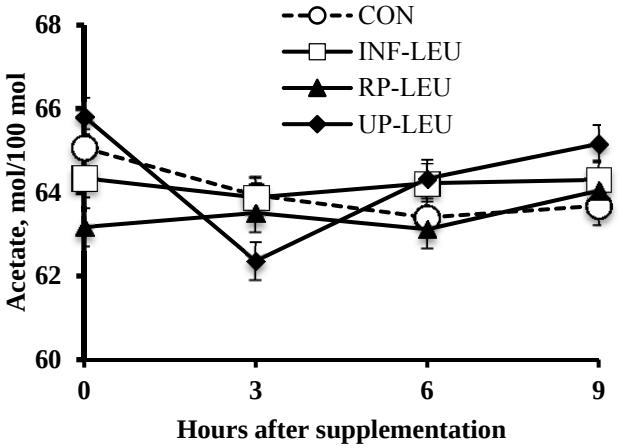
Lower molar proportions of acetate for UP-LEU than CON, RP-LEU, and INF-LEU at 3 h, and minimal effects at 0, 6, and 9 h indicates that supplementation of unprotected L-Leu altered microbial fermentation, whereas rumen-protected L-Leu has minimal effect. However, greater molar percentages of isovalerate in rumen fluid of lambs receiving both UP-LEU and RP-LEU compared to CON and INF-LEU indicates that the rumen-protected L-Leu was partially available to rumen microorganisms. According to Lui et al. (2009), anaerobic bacterial degradation of Leu, Ile, and Val in the rumen results in the production of isovaleric, 2-methylbutyric, and isobutyric acids, respectively. Our preliminary research (data not presented) demonstrated that 75% of the L-Leu in the rumen-protected Leu products escaped microbial degradation in an in situ study.



Figures 1. Plasma Leu and Ile concentrations of lambs receiving supplements (Table 2) containing no added Leu (CON), rumen-protected L-Leu (RP-LEU), unprotected L-Leu (UP-LEU), or post-ruminal infused of L-Leu (INF-LEU). Effects for Leu: treatment × h ($P < 0.01$), treatment ($P < 0.01$), h ($P < 0.01$); effects for Ile: treatment × h ($P = 0.02$), treatment (P = 0.07), h ($P < 0.01$).

IMPLICATIONS

Although supplementation of the ruminally-protected leucine source was unable in elevate blood leucine levels, the observed antagonistic effects of post-absorptive leucine on blood isoleucine levels imply that the ruminally-protected leucine was absorbed by the gastrointestinal tract of sheep. However, altered rumen fermentation also demonstrates that the ruminally-protected leucine source was not entirely protected from rumen microorganisms.



Figures 2. Molar percentage of acetate in rumen fluid of lambs receiving supplements (Table 2) containing no added Leu (CON), rumen-protected L-Leu (RP-LEU), unprotected L-Leu (UP-LEU), or post-ruminal infused of L-Leu (INF-LEU). Effects were: treatment × h ($P < 0.01$), treatment ($P = 0.09$), h ($P < 0.01$).

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Table 3. Plasma Leu and Ile concentrations and rumen pH, VFA and NH_3concentrations of lambs supplemented with different sources of L-Leu

	Treatments[1]					*P*-value		
	CON	UP-LEU	RP-LEU	INF-LEU	SEM	TRT	Hour	TRT × Hour
Plasma AA[2], μM								
Leu	61.2	74.2	69.0	162	5.29	< 0.01	< 0.01	< 0.01
Ile	58.5	57.2	52.5	51.9	1.73	0.07	< 0.01	0.02
Rumen fluid								
pH	6.48	6.54	6.57	6.58	0.04	0.42	< 0.01	0.90
NH_3, m*M*	4.01	3.52	3.30	3.42	0.27	0.40	< 0.01	0.52
Total VFA, m*M*	120	107	117	114	4.97	0.34	< 0.01	0.60
VFA, mol/100 mol								
Acetate[3]	64.0	64.4	64.2	63.5	0.22	0.09	< 0.01	< 0.01
Propionate	15.4	14.0	13.6	14.2	0.60	0.30	< 0.01	0.34
Isobutyrate	1.08	1.17	2.10	1.08	0.40	0.36	0.49	0.43
Butyrate	17.4	17.5	16.6	18.4	0.42	0.10	< 0.01	0.70
Isovalerate	1.14	1.91	3.12	1.11	0.26	< 0.01	0.07	0.20
Valerate	1.01	0.97	1.21	0.97	0.07	0.12	< 0.01	0.72
Acetate:Propionate	4.33	4.71	4.96	4.64	0.30	0.54	< 0.01	0.40

[1]Treatments were supplements (Table 2) containing no added Leu (CON), rumen-protected L-Leu (RP-LEU), unprotected L-Leu (UP-LEU), or post-ruminal infused of L-Leu (INF-LEU).
[2]Effects of treatment × h are presented in Fig. 1.
[3]Effects of treatment × h are presented in Fig. 2.

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Performance and net energy in high and low RFI beef cattle

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ABSTRACT: The objective of this study was to relate feed efficiency to performance and net energy in beef cattle. To identify animals with greater or lesser feed efficiency, 98 weaned Angus cross beef calves (71 steers and 27 heifers) were fed individually for 56 days. Feed offered and refused were measured daily, body weights were taken at 14 day intervals, and ultrasound measures (longissimus muscle area and subcutaneous fat over the 12^{th}-13^{th} ribs) were taken at the beginning, middle and end of the trial. Feed was delivered twice a day, on an *ad libitum* basis. Residual feed intake (RFI) was determined as the residual of the regression of DMI on mid-test $BW^{0.75}$ and ADG. High and low RFI groups were defined as > 0.5 SD above or below zero, respectively, with intermediate animals classified as medium RFI. As expected, RFI groups had similar initial and final BW and ADG, and different DMI, gain:feed and RFI ($P < 0.001$). Fat gain, protein gain, and recovered energy (RE) were not different between RFI groups, although subcutaneous fat over the 12^{th} -13^{th} rib was 0.19 cm higher in high RFI than low RFI cattle ($P = 0.012$). Heat energy (HE), defined as the difference between metabolizable energy intake (MEI) and RE was lower in low RFI cattle ($P < 0.001$). Estimated NEm requirement ($Mcal/kg^{0.75}$) was lower in low than in high RFI cattle ($P = 0.001$). Overall heifers gained less than steers, however there were no sex x RFI class interactions. Low RFI cattle have similar weights and weight gains, but lower intakes and higher feed efficiencies as high RFI cattle. This may be partially due to decreased maintenance requirement and heat production.

Key words: beef cattle, efficiency, net energy, body composition, residual feed intake

INTRODUCTION

Feed costs account for up to 70% of the expenses in beef production (Herd et al., 2003), and the majority of those costs are attributed to maintaining mature cows (Gregory, 1972). There is great interest in genetic selection to improve feed efficiency, resulting in progeny that eat less without sacrificing growth performance (Richardson et al. 1998). The beef industry has selected for improved feed conversion ratio (gain:feed) at the expense of increased mature cow size and maintenance costs (Herd et al, 2003). Residual feed intake (RFI), defined as the residual of the regression of DMI on mid-test $BW^{0.75}$ and ADG (Koch et al., 1963; Arthur et al., 2001; Herd et al. 2003), has gained acceptance as a measure of feed efficiency that is independent of growth rate and mature body size. The usual statistical model is:

$$DMI = \beta_0 + \beta_1 \cdot BW^{0.75} + \beta_2 \cdot ADG + RFI$$

Previous work has shown that 1) RFI is moderately heritable ($h^2 \approx 0.40$) and 2) variation in RFI is directly related to differences in maintenance energy requirements (Castro-Bulle et al., 2007; Cruz et al., 2007). Maintenance energy requirement varies with level of feeding, and the effects of feed intake on maintenance energy expenditure probably contribute to variation in RFI (Sainz et al., 1995; Castro-Bulle et al., 2007; Oltjen et al., 2001, 2006). Since RFI is related to lower maintenance requirement and is independent of mature size, it can serve as a tool for selection for more efficient beef cows (Hafla et al., 2013).

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The objectives of this study were to; 1) identify high, medium and low RFI beef calves, and 2) determine differences in body composition, maintenance requirement and heat production between RFI groups. It is hypothesized that low RFI cattle have lower maintenance requirement and heat production than their high RFI counterparts.

MATERIALS AND METHODS

To identify high and low RFI animals, 98 weaned calves (predominantly Angus, 71 steers and 27 heifers, 263 ± 29 d of age) from the UC Davis herd underwent a 56 day feeding trial after a 21 day adaptation to diet and 7 day adaptation to individual feeding pens. Upon arrival to the feedlot, steers received one implant of Revalor-S® (Merck Animal Health, Madison, NJ). Animals were housed individually in 2.5 x 10 meter pens, with half of each pen under roof. Animals were fed twice daily, on an *ad-libitum* basis, a total mixed ration, and feed refused was measured once daily. Amount offered was adjusted every 3 days based on the previous 3 days' intake to maintain approximately 10% refusals. The diet was composed (as-fed basis) of 69.8% flaked corn, 7.9% distillers grains, 5.9% wheat hay, 5.9% sudan hay, 5.6% molasses, 1.17% urea, 1.8% fat, 0.13% magnesium oxide, 1.42% calcium carbonate, 0.32% trace mineral salt, and 0.015% Rumensin® (Elanco, Greenfield, IN). The diet was formulated to contain 2.983 Mcal/kg DM of ME, 2.017 Mcal/kg DM of NEm and 1.361 Mcal/kg DM of NEg. Shrunk (18 hour solid feed withdrawal) body weights were taken at 14 day intervals. Average daily gain (ADG) was estimated as the slope of the regression of BW on time. Real-time ultrasound measures of the longissimus muscle area

(REA) and subcutaneous fat over the 12th-13th ribs (BF) were taken on days 0, 28, and 56 of the trial. Those data were used along with the USDA Yield Grade and Perry and Fox (1997) equations (Table 1) to estimate empty body fat (EBF) and protein (EBP). Protein gain, fat gain, and RE were estimated as the slope of the regression of EBP, EBF, and RE (calculated from the equations in Table 1) on time. High and low RFI groups were defined as > 0.5 standard deviation above or below zero, respectively, with intermediate animals classified as medium RFI. Data were analyzed using ANOVA with RFI phenotype (high, medium, low), sex, and sex x RFI interaction as effects. Statistical analyses were conducted using Minitab (Minitab, Inc., State College, PA). The results were considered statistically significant at the 5% probability level.

RESULTS AND DISCUSSION

The equation to calculate RFI was:

$$DMI = -2.64 + 0.123\ BW^{0.75} + 0.854\ ADG + RFI;$$
$$R^2 = 0.80,\ s_{y.x} = 0.53$$

As expected, RFI groups had similar initial and final BW and ADG ($P > 0.05$), although heifers were lighter and gained less than steers ($P < 0.001$) (Table 2). RFI groups had different DMI, gain:feed and RFI ($P < 0.001$). Low RFI (more efficient) cattle consumed 1.2 kg/day (8%) less DMI than High RFI counterparts. Subcutaneous fat over the 12th -13th rib was 0.19 cm greater in high RFI than low RFI cattle ($P=0.012$), while ribeye area averaged 68 cm^2 and was not different between groups ($P > 0.05$). Previous work has found that low RFI cattle tend to gain less fat than high RFI cattle, with no difference in protein gain, HCW, dressing percent, KPH fat, ribeye area, marbling score or yield grade (Castro-Bulle et al., 2007). Despite a difference in 12th-13th rib fat, calculated fat gain, protein gain, fat: protein and RE were not significantly different between RFI groups ($P > 0.05$). Heifers gained less fat and protein than steers, therefore having lower RE ($P < 0.001$), however were not different in fat:protein ratio. Overall, heifers were lighter and gained less than steers but behaved similarly within RFI phenotype as there were no sex x RFI interactions. Heifers likely performed differently than steers due to sex, as well as the use of a growth promotant implant in the steers. Implants were not used in heifers since they were not intended for slaughter after completion of the trial.

Estimated NEm requirement ($Mcal/kg^{0.75}$) was lower in low than in high RFI cattle ($P = 0.001$). Low RFI cattle required 0.026 $Mcal/kg^{0.75}$ less NEm than high RFI cattle. Previous work has also shown that low RFI cattle have lower maintenance energy requirements (Castro-Bulle et al., 2007; Cruz et al., 2007). Heat energy (HE), defined as the difference between metabolizable energy intake (MEI) and RE (assuming that all calories not retained in the body were lost as heat) was lower in low RFI cattle ($P < 0.001$). High RFI cattle produced 0.046 Mcal/ $kg^{0.75}$ more HE than low RFI cattle. Steers and heifers did not differ in HE in Mcal/ $kg^{0.75}$, although heifers had lower total HE due to being lighter. Low RFI cattle have similar weights and weight gains, but lower intakes and higher feed efficiencies as high RFI cattle. Low RFI phenotype cattle are likely able to perform similarly with less feed due to decreased maintenance requirement and heat production.

IMPLICATIONS

Selection for feed efficiency that is not related to mature body size or growth rate is important for genetic improvement that will not increase the maintenance requirement of the cow herd. Residual feed intake has been accepted as a measure of feed efficiency that identifies animals that consume less than expected without a difference in body weight or average daily gain. The working hypothesis is that a lower maintenance requirement and heat production are the main physiological mechanisms which improve feed efficiency in low RFI cattle. In this study, cattle divergent for RFI had no difference in average daily gain, body weight, fat gain or protein gain. The increased calories consumed by high RFI cattle were used for increased maintenance requirement and heat production.

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Table 1. Summary of equations used to calculate recovered energy and maintenance requirement from ultrasound, weight, and intake data.

Item	Equation	Reference
Metabolic body weight (kg)	MBW= $BW^{0.75}$	Kleiber, 1947
Empty body weight (kg)	EBW = 0.917*BW - 11.39	Owens, et al., 1995
Yield Grade	YG = 2.50 + 0.98425*BF + 0.20*KPH + 0.0008379*HCW - 0.0496*REA	USDA, 2016
Hot carcass weight (kg)	HCW = 0.6905*EBW - 22.259	Lofgreen et al., 1962
Empty body fat (kg)	EBFkg = (0.351*EBW) + (21.6*YG) - 80.8	Perry & Fox, 1997
Empty body protein (kg)	EBPkg = (EBW - EBFkg)*0.2201	Garrett & Hinman, 1969
Recovered energy (Mcal)	RE = EBFkg*9.367 + EBPkg*5.686	NRC, 2000
Net energy for maintenance requirement (Mcal/$kg^{0.75}$)	NEm = DMI-(RE/ NEg ·kg DM) * NEm·kg DM	NRC, 2000

Table 2. Performance, body composition, and net energy in RFI groups and sex after 56 days *ad libitum* feeding.

	RFI			Sex			P-value		
Trait	High	Medium	Low	H	S	SD	RFI	Sex	Sex x RFI
Initial BW, kg	279.3	272.3	277.2	261.3	291.2	30.2	0.67	< 0.001	0.53
Final BW, kg	382.6	370.1	382.4	346.7	410.0	35.9	0.33	< 0.001	0.32
ADG, kg/d	1.844	1.747	1.878	1.524	2.122	0.238	0.11	< 0 .001	0.32
DMI, kg/d	9.06	8.17	7.84	7.63	9.08	0.866	< 0.001	< 0.001	0.16
Gain:feed	0.203	0.212	0.239	0.202	0.235	0.02	< 0.001	< 0.001	0.96
RFI, kg/d	0.590	-0.006	-0.634	0.014	-0.047	0.25	< 0.001	0.29	0.29
Ribeye area, cm^2	67.52	65.18	67.80	63.82	69.85	6.26	0.23	< 0.001	0.35
12^{th}-13^{th} rib fat, cm	0.94	0.84	0.75	0.85	0.84	0.18	0.002	0.81	0.09
Fat gain, kg/d	0.64	0.61	0.61	0.517	0.722	0.17	0.65	< 0.001	0.17
Protein gain, kg/d	0.22	0.21	0.23	0.179	0.258	0.05	0.26	< 0.001	0.62
Fat:protein	3.02	2.81	2.73	2.892	2.817	0.67	0.29	0.62	0.06
RE, Mcal/d	7.25	6.89	7.00	5.860	8.231	1.78	0.63	< 0.001	0.19
RE, Mcal $kg^{0.75}$	0.102	0.099	0.098	0.089	0.112	0.02	0.77	< 0.001	0.28
HE, Mcal/d	19.40	17.14	16.08	16.58	18.50	2.69	< 0.001	< 0.001	0.40
HE, Mcal $kg^{0.75}$	0.274	0.248	0.228	0.250	0.250	0.03	< 0.001	0.91	0.55
NEm, Mcal/$kg^{0.75}$	0.103	0.087	0.077	0.098	0.081	0.03	0.001	0.002	0.75

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Impact of maternal protein restriction in first-calf heifers during mid- to late-gestation on gene expression, feedlot performance, and carcass characteristics of progeny[1]

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ABSTRACT: Maternal nutrient restriction in beef cows impacts developmental processes in the fetus that may influence postnatal performance. This study investigated impacts of MP restriction in mid- and late-gestation on the transcriptome of neonatal muscle tissue and subsequent feedlot performance and carcass characteristics of progeny. One hundred eight Angus × Simmental heifers were blocked by BW and method of conception (AI or natural service, based on fetal age at ultrasound) and allocated to 12 pens in a randomized complete block design with a 2 × 2 factorial treatment structure including 2 stages of gestation (mid- and late-) and 2 levels of dietary protein (control [CON]; approximately 102% of MP requirements and restricted [R]; approximately 80% of MP requirements). Pens were randomly assigned to CON or R treatments within blocks during mid- and/or late-gestation. Heifers were removed from treatments after calving and managed as a common group. Within 48 h of birth, LM biopsy samples were collected from a subset of 3 male AI calves from each treatment combination for analysis of gene expression using RNA-Seq technology. Following weaning, calves were backgrounded for two weeks then finished in a GrowSafe feeding system on a common finishing diet. Individual carcass measurements were recorded. Genes found in pathways associated with muscle tissue development were upregulated ($P \leq 0.02$) in calves born to dams on the CON treatment throughout mid- and late-gestation. Genes involved in adipogenesis were upregulated in calves born to dams on the R-R treatment ($P = 0.05$). No differences were observed for calf BW, DMI, ADG, G:F, or residual feed intake (RFI) due to maternal nutritional treatments across the entire feeding period ($P > 0.10$). Hot carcass weight, adjusted 12th rib fat thickness, KPH, marbling score, and proportion of carcasses in each USDA quality grade were not influenced ($P > 0.10$) by maternal diet during gestation. Progeny of dams on the R treatment in late gestation had greater LM area ($P = 0.05$) vs. progeny from CON dams. There was a tendency ($P = 0.06$) for a mid- by late-gestation treatment interaction for yield grade, with lower yield grades in progeny from dams on CON-R or R-CON treatments vs. CON-CON. Differences in gene expression, animal performance, and carcass characteristics indicate MP restriction during mid- and late-gestation may impact developmental programming.

Key words: Beef cattle, MP restriction, gene expression

INTRODUCTION

The fetal origins hypothesis suggests that exposing the fetus to an adverse environment *in utero* leads to permanent programming of tissue function and increased risk of disease (Drake and Walker, 2004). Nutrient restriction during gestation in livestock may result in unfavorable postnatal growth, feed efficiency, as well as changes in body composition and meat quality (Wu et al., 2006). However, little research is available that evaluates how restriction of specific nutrients such as MP in the maternal diet affects developmental processes in beef cattle. Our hypothesis was that MP restriction in mid- and late-gestation would result in differential gene expression, reductions in postnatal growth and skeletal muscle, and increased carcass adiposity. Therefore, the objectives of this study were to investigate the impacts of MP restriction in mid- and late-gestation on the transcriptome of neonatal muscle tissue and subsequent feedlot performance and carcass characteristics of progeny.

MATERIALS AND METHODS

Animals and Experimental Design. All procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee. One hundred eight Angus × Simmental heifers were allocated to 12 pens in a randomized complete block design. Heifers were blocked by BW, method of conception (AI or clean-up through natural service) and fetal sex as determined by ultrasound. Treatments were arranged in a 2 × 2 factorial treatment structure with 2 levels of dietary protein: control (CON; approximately 102% of MP requirements) and restricted (R; approximately 80% of MP requirements) provided during 2 stages of gestation: mid- (mean d 148 through 216 of gestation) and late- (mean d 217 through parturition). The design resulted in 3 blocks with 4 pens per block. At the end of the mid- gestation period, half of the pens on the CON treatment were reassigned to the R treatment and half of the pens on the R treatment were reassigned to the CON treatment, resulting in four treatment combinations (CON-CON, CON-R, R-CON, and R-R). Each treatment combination was randomly assigned to one pen per block for a total of 3 pen replicates per treatment combination. Diets were based on calcium hydroxide treated wheat straw and concentrates, and were adjusted throughout gestation to maintain MP balance across treatments and account for increased nutrient requirements for the growing heifer and the developing fetus (NRC, 2000). Concentrate formulations between treatments were similar, except that porcine

[1]Research supported by the South Dakota Beef Industry Council

bloodmeal was added to the CON formulation to slightly exceed the MP requirement (Table 1). The NRC (2000) requirements for NE_m and NE_g were met in both dietary treatments, and diets were formulated to be isocaloric. Immediately after calving, heifers were removed from treatments and managed as a common group. Additional information about heifer and suckling calf performance through weaning was previously reported by Kincheloe et al. (2015).

Progeny Muscle Biopsies and Gene Expression Analyses. Biopsy samples (approximately 40 mg) were collected from the LM for analysis of gene expression on a subset of three male AI-bred calves from each treatment combination within 48 h of birth. Calves were pre-selected by choosing dams representative of treatment means for BW and BCS at the beginning of the study and at treatment crossover. An area approximately 12.7 cm^2 was shaved using surgical precision blades and scrubbed with a povidone-iodine solution, followed by a 70% alcohol solution. A total of 5 mL of lidocaine was injected in a circle of beads around the planned incision site. After local anesthesia was established, a 10 mm incision was made using a sterile No. 11 scalpel, and a BARD Magnum Reusable Core Biopsy System with a 12 G × 10 cm needle was used to collect muscle tissue (C.R. Bard, Inc., Tempe, AZ). Tissue was immediately removed from the biopsy needle and snap frozen in liquid N before storage at −80°C. The injection site was sprayed with Vetericyn antimicrobial topical spray (Vetericyn, Rialto, CA) and calves were closely monitored until fully recovered.

Total RNA was extracted from muscle samples using the miRNeasy Mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Purity of the RNA was evaluated by spectroscopy to ensure samples had an optical density 260:280 greater than 2.0. All purified total RNA samples were stored at −80°C prior to gene expression evaluation. A transcriptome library was created from each of the individual RNA samples and sequenced (2 sequencing lanes per sample) with an Illumina HiSeq 2000 genome analyzer (Illumina Inc., San Diego, CA) by paired-end sequencing. Transcripts were mapped to the bovine genome sequence for gene identification, and number of transcript counts were used as a measure of gene expression. Transcriptome library preparation and sequencing was completed by LC Sciences, Houston, TX.

Weaning and Feedlot Management. One hundred three steer and heifer calves were weaned on October 6, 2014. All calves were backgrounded on high quality grass hay and dried distillers grains for 14 d at the SDSU Cottonwood Range and Livestock Field Station near Philip, SD and then shipped approximately 430 km to the University of Nebraska-Lincoln West Central Research and Extension Center in North Platte, NE.

Calves were allocated to four feedlot pens based on sex and method of conception (AI or clean-up through natural service) and adapted to a final finishing diet in 54 d using 3 step-up diets containing 35, 25, and 14% roughage, fed for 7, 7, and 40 d, respectively. Calves remained within these four groups and were placed in a GrowSafe system to collect individual feed intake data beginning November 22 for AI-bred calves and December 13 for bull-bred calves due to space limitations in the facility. All calves received the same diet whether they were being fed in standard feedlot pens or in the GrowSafe system. The final finishing diet was composed of 40% wet corn gluten feed (Sweet Bran, Cargill Inc., Blair, NE), 48% dry-rolled corn, 7% grass hay, and 5% supplement containing 58.25% ground corn, 29.57% limestone, 5.59% iodized salt, 4.65% ammonium chloride, 0.93% trace mineral mix, 0.31% Rumensin (Elanco Animal Health, Greenfield, IN), 0.25% thiamine, 0.24% Tylan 40 (Elanco Animal Health, Greenfield, IN), and 0.21% Vitamins A, D, and E. Nutrient composition was 69.15% DM, 15.8% CP, 1.98 Mcal/kg NE_m, and 1.33 Mcal/kg NE_g. Initial feedlot weights were collected on November 20 and 21, 2014, following 10 d of adaptation in the GrowSafe system. Steers received an initial feedlot implant of Revalor-IS (80 mg trenbolone acetate and 16 mg estradiol), and heifers received Revalor-IH (80 mg trenbolone acetate and 8 mg estradiol; Merck Animal Health, Madison, NJ). Individual BW were recorded and cattle were re-implanted with Revalor-200 (200 mg trenbolone acetate and 20 mg estradiol; Merck Animal Health, Madison, NJ) and dewormed with Agrimectin (Agri Laboratories Ltd., St. Joseph, MO) on March 3, 2015.

Harvest and Carcass Evaluation. Cattle were fed and managed to maintain health and achieve an industry average endpoint at harvest. The AI-bred steers and heifers were shipped approximately 100 km to Tyson Fresh Meats in Lexington, NE on May 13, 2015, and bull-bred steers and heifers were shipped to the same processing facility on June 3, 2015. Individual carcass measurements included HCW, LM area, 12^{th} rib backfat, and estimated percentage of KPH. Yield grade was calculated according to USDA guidelines, and marbling score and carcass maturity were recorded and used to determine quality grade. Cattle were not weighed prior to shipping to the processing facility to reduce incidence of bruising and injury; therefore, final live BW was determined as HCW divided by 0.625 (assumed dressing percentage).

Statistical Analysis. Raw individual RNA sequence reads were aligned to the reference beef cattle genome (Ensembl UMD3.1) using the software package Tophat (v2.0.12), with the abundance normalized and evaluated in Fragments Per Kilobase of transcript per Million mapped reads (FPKM) using the Cuffdiff module of Cufflinks (v2.2.1). Pairwise comparisons were conducted at the gene level, with differentially expressed genes analyzed for Gene Ontology (GO) terms and KEGG Pathways (using GAGE v2.18 of Bioconductor). All statistical analyses for differential expression of genes were conducted by LC Sciences, Houston, TX.

Residual feed intake (RFI) was calculated using PROC GLM of SAS (SAS Institute Inc., Cary, NC). Feedlot performance measures (DMI, ADG, G:F, and RFI) and carcass characteristics (HCW, LM area, 12^{th} rib backfat, KPH, yield grade, and marbling score) were analyzed using the MIXED procedure of SAS to determine differences due to the fixed effects of maternal nutritional treatment during mid- and late-gestation and their interaction. Initial, reimplant, and ending BW were analyzed as repeated measures with time as the repeated effect. Individual animal

was considered random and used as the experimental unit for all data analysis. Fixed effects of calf sex and method of conception (AI or natural service) were also included in the model. Least squares means and SEM were estimated and separated by LSD (i.e. the PDIFF option) if the tests of fixed effects were significant. The influence of maternal nutritional treatments on proportion of cattle assigned to each USDA yield and quality grade were analyzed using a binary distribution in the GLIMMIX procedure of SAS. The fixed effects of mid- and late- gestation treatments and their interaction were included as fixed effects and individual animal was considered random and used as the experimental unit. Denominator degrees of freedom were estimated using the Kenward-Roger option. Least squares means and SEM of the proportions were estimated using the ILINK option and separated as described above. All tests were considered significant at $P \leq 0.05$, with tendencies considered at $P < 0.10$.

RESULTS AND DISCUSSION

Gene Expression. A total of 652, 81, and 191 genes were differentially expressed between the CON-CON dietary treatment and other treatment combination (CON-R, R-CON, and R-R, respectively. There were notably fewer gene expression differences between progeny from dams experiencing MP restriction in mid- gestation and throughout gestation (R-CON and R-R) compared to those on the control treatment throughout gestation (CON-CON). In a study examining gene expression profiles in fetal LM at various stages of myogenesis and muscle maturation, Lehnert et al. (2007) reported the most marked increase or decrease in gene expression was observed in late gestation. Therefore, it is possible that gene expression was more responsive to maternal nutritional treatments in our study during that time (i.e. gene expression differences being greatest in the CON-R treatment). It also appears that genetic adaptation to the MP restriction may have occurred in progeny from dams that were restricted throughout gestation (R-R).

Differentiation of mesenchymal stem cells to myogenic, adipogenic, or fibrogenic cells is a competitive process shaped by a number of regulatory factors (Yan et al., 2013). Genes in pathways associated with muscle tissue development (ZFAND5, MYL3, HOMER1, PLN) were upregulated ($P \leq 0.02$) in calves whose dams were on the control diet throughout gestation (CON-CON) as opposed to dams restricted throughout gestation (R-R) or only in late gestation (CON-R). Interestingly, genes related to positive regulation of fat cell development were upregulated in calves from dams on the R-R treatment compared with CON-CON ($P = 0.05$). One of the differentially expressed genes was PPARγ, known to stimulate adipocyte gene transcription and drive adipogenesis. This process is also controlled by the Wnt signaling pathway, which was upregulated ($P = 0.009$) in the CON-R progeny compared with CON-CON progeny. These results indicate that MP restriction, particularly in late gestation, may have shifted gene expression in favor of adipogenosis rather than myogenesis.

Genes involved in fatty acid, lipid, and triglyceride metabolic processes (DGAT2, LPL, GPAM, LIPE) were upregulated ($P < 0.0001$) in calves from the R-R treatment compared with the CON-R treatment, providing evidence that MP restriction throughout mid- and late-gestation may stimulate lipogenesis. Zhu et al. (2006) restricted pregnant ewes at 50% of requirements from d 28-78 of gestation, and reported that intramuscular triglyceride content (IMTG) was increased in skeletal muscle of lambs from nutrient-restricted (NR) ewes. Additionally, reductions in insulin sensitivity and glucose utilization in skeletal muscle of NR progeny suggested that lambs would be predisposed to obesity and diabetes. In our study, MP restriction in late gestation (CON-R) showed downregulation ($P = 0.002$) of genes involved in the KEGG pathway for insulin signaling compared to the control treatment (CON-CON). Overall, differentially expressed genes in our study provide additional evidence that maternal nutrient status in mid- and late-gestation can impact programming of muscle and fat tissues and metabolic processes in beef cattle.

Feedlot Performance. There were no differences in initial, middle, or ending BW ($P > 0.10$; mean 268 ± 2.6, 417 ± 4.7, and 573 ± 4.9 kg, respectively). Overall DMI, ADG, G:F, and residual feed intake (RFI) did not differ ($P > 0.16$) due to maternal nutritional treatment in mid- or late-gestation (Table 2). Progeny born to dams restricted in late-gestation had greater ADG and G:F from the re-implant date through slaughter than progeny from dams on the CON treatment in late gestation ($P = 0.03$), with no differences ($P > 0.10$) in DMI or RFI. Summers et al. (2015) reported that ADG during the initial feeding period through reimplant tended to be greatest for calves born to dams fed meadow hay in late gestation and provided no supplement (CON) and lowest for calves born to dams receiving a dried corn gluten feed-based supplement, although there were no differences over the entire feeding period. Stalker et al. (2006) reported no differences in feedlot performance in calves from dams fed the equivalent of 0.45 kg of 42% CP supplement vs. those fed no supplement.

Carcass Characteristics. Hot carcass weight, adjusted 12th rib fat thickness, KPH, marbling score, and proportion of carcasses in each USDA quality grade were not influenced ($P \geq 0.18$) by maternal diet during gestation (Table 2). Longissimus muscle area was greater ($P = 0.05$) for calves whose dams were restricted in late gestation vs. those on the control treatment (92.29 ± 1.090 vs. 89.31 ± 1.072 cm^2). In contrast, Underwood et al. (2010) found no differences in LM area of steers whose dams were placed on improved pasture (IP) or native range (NR) in mid- to late-gestation; however, heavier HCW and increased 12th rib fat thickness were observed in progeny from IP dams. Mohrhauser et al. (2015) reported that restricting dams to 80% of NE_m did not influence progeny HCW, LM area, KPH, or marbling score; however, tendencies for decreased 12th rib backfat and lower final USDA yield grade were observed in progeny from dams in a negative energy status in mid- gestation.

There was a tendency ($P = 0.06$) for a mid × late-gestation treatment interaction for yield grade. No difference ($P > 0.10$) was observed for yield grade among progeny from dams assigned to the CON-CON or R-R

treatment throughout gestation (3.03 ± 0.136 and 2.76 ± 0.141, respectively). However, a restriction imposed in either mid- (R-CON) or late- (CON-R) gestation resulted in a lower yield grade ($P < 0.10$) for progeny from those dams (2.69 ± 0.141 and2.59 ± 0.140, respectively) compared with progeny whose dams were on the CON-CON treatment. There was also a mid- × late- gestation treatment interaction ($P = 0.05$) for proportion of progeny in the yield grade 3 category, with greater proportions of progeny from the CON-CON and R-R treatments (62.98 ± 9.492 and 72.03 ± 9.142%, respectively) than from the CON-R treatment (38.44 ± 9.749%), with the R-CON treatment intermediate and similar to all other treatments (56.01 ± 10.150%). There was also a tendency ($P = 0.07$) for a mid- × late-gestation treatment interaction on proportion of progeny in the yield grade 2 category, with the greatest proportion of progeny from the CON-R treatment (34.57 ± 9.591%) achieving yield grade 2, while the R-CON proportion was intermediate and similar to all other treatments (19.96 ± 8.148%). The CON-CON and R-R treatments had the lowest proportions of yield grade 2 carcasses (11.09 ± 6.112 and 11.98 ± 6.573%, respectively). Similar to results observed in gene expression analysis, the most notable differences among treatments appear to be between CON-CON vs. CON-R progeny, suggesting that MP restriction late in gestation has the greatest impact on developmental processes.

Differential gene expression in CON vs. R treatments support the hypothesis that MP restriction in mid- and late-gestation influenced gene expression. However, most phenotypic responses for feedlot performance and carcass characteristics were non-significant or inconsistent with expected results; therefore, we reject this portion of the hypothesis.

IMPLICATIONS

Differential gene expression among neonatal progeny indicated that maternal MP restriction during mid- and late-gestation may alter developmental programming of muscle and adipose tissues and metabolic processes in beef cattle. Although genetic differences did not consistently translate to corresponding phenotypic responses, MP restriction in late- gestation influenced feed efficiency, feedlot BW gain, and LM area. Inconsistent responses may be due to timing and duration of nutrient restriction, influence of MP as opposed to energy restriction, and environmental factors. It is also plausible that calves are able to recover from restriction during development when exposed to an unrestricted nutritional environment postnatally.

These results suggest further investigation is warranted to elucidate potential influences of timing and interactions among nutrients restricted during fetal development that may impact the neonatal transcriptome, animal performance, and carcass characteristics.

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Table 1. Dietary components and nutrients supplied to heifers receiving a control (CON; approximately 102% of MP requirement) or restricted (R; approximately 80% of MP requirement supplied) diet in mid- or late gestation based on NRC (2000) calculations

	Mid-gestation		Late-gestation	
	CON	R	CON	R
Ingredient	---- % DM basis ----			
Straw	60.5	60.3	56.5	56.1
Ground corn	4.7	4.6	10.6	10.5
Ground corn cobs	13.7	13.7	12.3	12.6
Energy Booster 100®	5.3	5.5	8.0	8.2
Urea	0.7	0.8	0.6	0.8
Bloodmeal	1.8	-	1.6	-
Glycerin	13.3	15.1	10.4	11.7
	---- Nutrient composition of diet predicted by NRC (2000) ----			
Bacterial N balance, g/d	5	5	5	8
MP, %	102.1	78.7	101.4	79.6
NE_m, Mcal/kg	0.26	0.26	0.28	0.28
NE_g, Mcal/kg	0.14	0.14	0.16	0.16

Table 2. Main effect least square means for effect of maternal MP restriction in mid- and late- gestation on progeny feedlot performance and carcass characteristics[1]

	Mid-gestation Period		Late-gestation Period		*P*-value[2]	
Item	CON	R	CON	R	Mid	Late
Feedlot Performance						
Initial period						
ADG, kg	1.31 ± 0.028	1.27 ± 0.029	1.31 ± 0.028	1.27 ± 0.029	0.269	0.391
DMI, kg	9.95 ± 0.162	9.86 ± 0.165	9.92 ± 0.163	9.89 ± 0.165	0.664	0.875
G:F	0.132 ± 0.003	0.129 ± 0.003	0.132 ± 0.003	0.129 ± 0.003	0.536	0.463
RFI	-0.030 ± 0.151	-0.087 ± 0.154	-0.042 ± 0.152	-0.075 ± 0.154	0.783	0.876
Reimplant period						
ADG, kg	2.57 ± 0.041	2.60 ± 0.042	2.53 ± 0.413	2.65 ± 0.042	0.607	0.029
DMI, kg	10.14 ± 0.143	10.33 ± 0.146	10.26 ± 0.143	10.21 ± 0.146	0.326	0.815
G:F	0.254 ± 0.005	0.252 ± 0.005	0.246 ± 0.005	0.260 ± 0.005	0.725	0.025
RFI	-0.128 ± 0.141	0.012 ± 0.143	0.053 ± 0.141	-0.169 ± 0.143	0.471	0.253
Overall						
ADG, kg	1.83 ± 0.22	1.82 ± 0.022	1.81 ± 0.022	1.84 ± 0.022	0.668	0.290
DMI, kg	10.09 ± 0.138	10.16 ± 0.140	10.14 ± 0.138	10.11 ± 0.140	0.714	0.850
G:F	0.182 ± 0.002	0.179 ± 0.002	0.179 ± 0.002	0.183 ± 0.002	0.380	0.225
RFI	-0.044 ± 0.116	0.063 ± 0.119	0.063 ± 0.117	-0.044 ± 0.119	0.504	0.505
Carcass Characteristics						
HCW, kg	359±3.4	353±3.5	354±3.4	360±3.5	0.382	0.193
Adj. 12th rib backfat, cm	1.39±0.070	1.33±0.072	1.41±0.070	1.31±0.072	0.593	0.303
LM area, cm^2	90.89±1.069	90.71±1.090	89.31±1.072	92.29±1.090	0.903	0.045
KPH, %	2.25±0.065	2.18±0.066	2.18±0.065	2.25±0.067	0.419	0.440
Yield grade	2.81±0.100	2.72±0.102	2.86±0.100	2.67±0.102	0.536	0.175
Marbling score[3]	531±11.1	526±11.3	534±11.1	522±11.3	0.742	0.460
USDA Quality Grade[4]						
All Choice, %	81.1 ± 5.821	86.2 ± 4.983	80.7 ± 5.914	86.4 ± 4.895	0.508	0.459
Prime, %	18.95±5.821	13.85±4.983	19.29±5.914	13.58±4.895	0.508	0.459
USDA yield grade[5]						
Yield grade 2, %	20.43±6.104	15.56±5.290	14.99±5.115	21.14±6.286	0.548	0.449
Yield grade 3, %	50.76±7.238	64.42±7.025	59.55±6.978	55.91±7.554	0.185	0.725
Yield grade 4, %	20.64±5.682	17.15±5.587	23.05±5.972	15.24±5.191	0.664	0.332

[1] Dams received a control (CON; approximately 102% of MP requirement) or restricted (R; approximately 80% of MP requirement supplied) diet based on calcium hydroxide treated wheat straw and concentrates in mid- or late gestation

[2] Probability of a greater F for the tests of fixed effects

[3] 400 = Small[00]; 500 = Modest[00]; 600 = Moderate[0]

[4] No animals received a select quality grade

[5] GLIMMIX analysis failed to converge for USDA yield grade 1 (n = 1) or yield grade 5 (n = 2)

PHYSIOLOGY AND ENDOCRINOLOGY

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Insulin-associated and insulin-independent impacts of β adrenergic agonists and pro-inflammatory cytokines on glucose metabolism in primary rat soleus muscle.

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ABSTRACT: Recent studies show that catecholamines and pro-inflammatory cytokines may help regulate skeletal muscle growth and metabolism even at sub-stress levels. The objective of this study was to determine the acute effects of β1 and β2-specific adrenergic agonists as well as TNFα and IL-6 on muscle glucose uptake and oxidation under basal and insulin-stimulated conditions. Primary soleus muscle was collected from adult Sprague-Dawley rats, separated tendon-to-tendon into 25-45 mg strips, and incubated in KHB spiked with or without insulin, and/or ractopamine HCl (β1 agonist), zilpaterol HCl (β2 agonist), TNFα, and IL-6. Glucose uptake was determined from cellular content of [^{3}H]-2-deoxyglucose after 20 min. Glucose oxidation of [^{14}C-U] glucose was determined after 2 h. Phospho-Akt/total Akt (**p-Akt/Akt**) was determined from protein isolated after 1 h. Compared to muscle incubated in un-spiked (basal) media, incubation with insulin increased ($P < 0.05$) glucose uptake by ~47%, glucose oxidation by ~32%, and p-Akt/Akt by ~238%. Muscle incubated with β2 agonist exhibited ~20% less ($P < 0.05$) glucose uptake but ~32% greater ($P < 0.05$) glucose oxidation than basal. Moreover, incubation with β2 agonist+insulin increased ($P < 0.05$) glucose oxidation and p-Akt/Akt over insulin alone. Muscle incubated with β1 agonist did not differ from basal for any output. Likewise, β1 agonist+insulin incubations did not differ from insulin alone. Glucose oxidation was ~23% and ~33% greater ($P < 0.05$), respectively, in muscle incubated with TNFα and IL-6 compared to basal, yet glucose uptake and p-Akt/Akt did not differ. Glucose uptake, glucose oxidation, and p-Akt/Akt were similar among muscle incubated with TNFα+insulin, IL-6+insulin, and insulin alone. In addition, glucose oxidation in muscle incubated with TNFα+insulin and IL-6+insulin did not differ from TNFα alone or IL-6 alone. These results show that acute β2 stimulation had opposite effects on glucose uptake and glucose oxidation in muscle, and that acute β1 stimulation had no evident impact on muscle metabolism. Moreover, β2 stimulation was synergistic with insulin, as glucose oxidation and Akt phosphorylation were greater with the two products together than with either individually. Lastly, acute stimulation with TNFα or IL-6 increased glucose oxidation rates independently of insulin or Akt phosphorylation. Together, our findings demonstrate that adrenergic and inflammatory mediators can have insulin-associated or insulin-independent effects on glucose metabolism and that these effects may differ for glucose uptake and oxidation.

Key words: β-agonist, glucose metabolism, stress hormones

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INTRODUCTION

Skeletal muscle makes up ~40% of total body mass but accounts for >80% of insulin-stimulated glucose utilization (DeFronzo et al., 1981; Brown, 2014). In addition to insulin, muscle metabolism appears to be influenced by adrenergic and cytokine activity (Glund et al., 2007; Pillon et al., 2013; Fernandes et al., 2014). Adrenergic activity in muscle is mediated primarily by β receptors. β2 receptors are the most prevalent, but β1 receptors and to a lesser extent β3 receptors are also present (Kim et al., 1991). Performance studies (Lopez-Carlos et al., 2012) have led to the development of isomer-specific adrenergic growth promoters (Johnson et al., 2014), but far less is known about their effects on metabolism. Likewise, pro-inflammatory cytokines have complex metabolic effects that are only beginning to be understood. Inflammation is known to cause insulin resistance (Marette et al., 2014), but recent studies show that two major pro-inflammatory cytokines, TNFα and IL-6, may stimulate glucose metabolism in muscle (Glund et al., 2007; Gray and Kamolrat, 2011; Remels et al., 2015). Insulin activates a pathway that ultimately translocates the glucose transporter Glut4 to the cell membrane where it is imbedded (James et al., 1988). A major step in this pathway is phosphorylation of the kinase Akt, which is often used to indicate insulin sensitivity. Insulin-stimulated glucose uptake and oxidation are often assumed to be proportional, but we postulate that this relationship may not be maintained when adrenergic factors or cytokines are present. Moreover, adrenergic factors and cytokines may influence glucose metabolism via insulin-independent effects. Our objective was to determine the impact of β1 and β2-specific adrenergic agonists as well as TNFα and IL-6 on muscle glucose uptake and oxidation. Furthermore, we sought to determine whether these effects were insulin-associated or insulin-independent by incubating muscle strips with each factor alone or with insulin.

MATERIALS AND METHODS

Animals and tissue isolation

The following experiments were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln. Animal studies were performed at the University of Nebraska-Lincoln Animal Science Complex which is accredited by the American Association for Accreditation of Laboratory Animal Care.

Soleus muscles were collected tendon-to-tendon from mature Sprague-Dawley rats (female 252.86 ± 14.93

g, male 378 ± 15.72 g) after decapitation under heavy anesthesia (isoflurane) and used to measure glucose uptake, glucose oxidation, and Akt phosphorylation (n = 10, 9, and 8 rats, respectively). Males and females were spread evenly across all groups. Isolated muscles were washed in ice-cold phosphate buffered saline (PBS), and each muscle was dissected longitudinally into 25-45 mg strips (2 technical replicates per condition for each rat). Strips were then pre-incubated for 1 h at 37°C in gassed (95% O_2, 5% CO_2) Krebs-Henseleit bicarbonate buffer (KHB, pH 7.4; 0.1% bovine serum albumin; Gibco Life, Grand Island, NY) spiked with treatment (see below) and 5mM glucose and then washed for 20 min in treatment-spiked KHB with no glucose. Pre-incubation and wash media for glucose uptake experiments also contained 35 mM and 40 mM mannitol, respectively. To determine glucose uptake, glucose oxidation, and Akt phosphorylation, muscle strips were then incubated in treatment-spiked KHB under the below conditions.

Pre-incubation, wash, and incubation media were spiked with the following treatments: none (basal), insulin (5 mU/ml Humulin-R), β1 agonist (10 μM ractopamine HCl), β2 agonist (0.5 μM zilpaterol HCl), β1+insulin, β2+insulin, TNFα (20 ng/ml hTNFα), IL-6 (1 ng/ml rIL-6), TNFα+insulin, and IL-6+insulin. All additives were purchased from Sigma-Aldrich (St. Louis, MO).

Glucose uptake

Glucose uptake rates were determined by incorporation of [^{3}H]2-deoxyglucose as previously described (Jacob et al., 1996), with some modifications. After being washed, muscle strips were incubated at 37°C for 20 min in treatment-spiked KHB with 1 mM [^{3}H]2-deoxyglucose (300 μCi/mmol) and 39 mM [1-^{14}C] mannitol (1.25 μCi/mmol). Muscle strips were then removed, thrice washed in ice-cold PBS, weighed, and lysed in 2 M NaOH at 37°C for 1 h. Lysate was mixed with UltimaGold scintillation fluid and specific activity of ^{3}H and ^{14}C was measured by liquid scintillation with a Beckman-Coulter 1900 TA LC counter (Brea, CA). Specific activity of the media was likewise determined in triplicate from 10-μl aliquots mixed with 500 μl distilled water. All radioactive compounds and scintillation fluids were purchased from Perkin-Elmer (Waltham, MA).

Glucose oxidation

Glucose oxidation rates were determined by oxidation of [^{14}C-U]D-glucose as previously described (Henriksen and Tischler, 1988), with some modifications. After being washed, muscle strips were placed in sealed dual-well chambers and incubated at 37°C for 2 h in treatment-spiked KHB with 5 mM [^{14}C-U] D-glucose (0.25 μCi/mmol). In the adjacent well, 2M NaOH was placed to capture CO_2. After 2 h, chambers were cooled at -20°C for 2 min, 2M HCl was added to the media through the rubber seal, and chambers were placed at 4°C for 1 h to release bicarbonate-bound CO_2 from the media. Chambers were then opened and muscle strips were washed and weighed. NaOH was collected and mixed with UltimaGold scintillation fluid to determine specific activity of $^{14}CO_2$ via liquid scintillation. Specific activity of media was also determined.

Phosphorylated Akt

Akt phosphorylation was determined by the proportion of phosphorylated Akt to total Akt (p-Akt/Akt) as previously described (Morley et al., 2015). Muscle strips were incubated in treatment-spiked KHB with 5 mM glucose at 37°C for 1 h and then snap-frozen in liquid nitrogen and stored at -80ºC.

Snap-frozen muscle was thoroughly homogenized in 200 μl of RIPA Buffer containing recommended concentrations of Protease and Phosphatase Inhibitor (Thermo Fisher, Carlsbad, CA). Homogenates were then sonicated and centrifuged (14,000 x *g*; 5 min at 4ºC), and supernatant was collected. Total protein concentrations were determined by Pearce BCA Assay (Thermo Fisher). Protein samples were combined with BioRad 4x Laemmli Sample Buffer (BioRad, Hercules, CA) and incubated at 95ºC for 5 min. Protein was loaded into a 15-well gel at 35 μg/well, separated by SDS-page and then transferred to polyvinylidene fluoride low florescence membranes(BioRad). Membranes were incubated in odyssey block (Li-Cor Biosciences, Lincoln, NE) solution for 1 h at room temperature and then washed with 1X TBS-T (20 mM Tris-HCL+ 150 mM NaCl + 0.1% Tween 20). Membranes were subsequently incubated overnight at 4ºC in rabbit anti-pAkt (1:2,000) or rabbit anti-Akt (1: 1,000) antibodies for one hour (Cell Signaling, Danvers, MA) diluted in odyssey block + 10μL Tween 20. An IR800 goat anti-rabbit IgG secondary antibody (1:10,000; Li-Cor) was applied for 1 h at room temperature, imaged on an Odyssey scanner and analyzed with Image Studio Lite Software (Li-Cor).

Statistical analysis

All data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, Cary, NC). Data were analyzed as five separate experiments, each with its own basal and insulin-only conditions: glucose uptake/ adrenergic factors (n = 10), glucose uptake/ cytokines (n = 10), glucose oxidation/ adrenergic (n = 9), glucose oxidation/ cytokine (n = 9), and Akt phosphorylation (n = 8). A separate muscle strip from each rat was exposed to each condition in the experiment. Data are presented and means ± standard error.

RESULTS

Glucose uptake and oxidation

Incubation of muscle with insulin increased ($P < 0.05$) glucose uptake in both the adrenergic (Figure 1) and cytokine (Figure 2) experiments. Likewise, glucose oxidation was increased ($P < 0.05$) by insulin compared to basal in adrenergic (Figure 3) and cytokine (Figure 4)

experiments. When muscle was incubated with β1 agonist alone, glucose uptake and oxidation did not differ from basal. Moreover, when muscle was incubated with β1+insulin, glucose uptake and oxidation did not differ from incubation with insulin alone. Incubation of muscle with β2 agonist decreased ($P < 0.05$) glucose uptake but increased ($P < 0.05$) glucose oxidation compared to basal. Glucose uptake did not differ between muscle incubated with β2+insulin and insulin alone, but glucose oxidation was greater ($P < 0.05$) in muscle incubated with β2+insulin than insulin alone or β2 agonist alone. Glucose uptake in muscle incubated with TNFα or with IL-6 did not differ from basal, and in muscle incubated with TNFα+insulin or IL-6+insulin did not differ from insulin alone. However, glucose oxidation in muscle incubated with TNFα or with IL-6 was greater ($P < 0.05$) than basal and in fact was not different from insulin alone. Interestingly, glucose oxidation in muscle incubated with TNFα+insulin or with IL-6+insulin did not differ from insulin alone, TNFα alone, or IL-6 alone.

Akt phosphorylation

Muscle incubated with insulin exhibited 4.26-fold greater ($P < 0.05$) p-Akt/Akt than basal (Figure 5), but muscle incubated with β1, β2, TNFα, or IL-6 alone did not differ from basal. Muscle incubated with β1+insulin did not differ in p-Akt/Akt from muscle incubated with insulin alone, but muscle incubated with β2+insulin was greater ($P < 0.05$) in p-Akt/Akt than insulin alone. Conversely, p-Akt/Akt in muscle incubated with TNFα+insulin or with IL-6+insulin was less ($P < 0.05$) than insulin alone and, in fact, IL-6+insulin did not differ from basal.

DISCUSSION

In this study, we show that both adrenergic and pro-inflammatory stimulation can acutely impact basal and insulin-stimulated glucose metabolic rates in skeletal muscle. Adrenergic effects were isomer-specific, as β1 stimulation had no discernable effect, but β2 stimulation impaired basal glucose uptake, increased basal oxidation, and was synergistic with insulin. Cytokines appeared to directly stimulate glucose oxidation and impair insulin-stimulated Akt phosphorylation, but had no effect on glucose uptake rates. These data indicate that stress factors may initially benefit glucose metabolism, particularly glucose oxidation, despite apparent changes in insulin sensitivity.

Acute β2-specific adrenergic stimulation had differing effects on glucose uptake and oxidation rates in the absence of insulin, as uptake was impaired but oxidation was enhanced. Total oxidative metabolic rates are believed to remain static but individual substrate oxidation may vary (Hay et al., 1983), and our findings show that fractional oxidation of glucose increases substantially during β2 stimulation. Indeed, this may help to explain smaller fractional glucose oxidation rates in growth-restricted fetuses (Limesand et al., 2007), which we previously found to have reduced skeletal muscle β2 receptors (Yates et al., 2012). In addition to its own direct effects, β2 stimulation appeared to synergistically enhance glucose oxidation with insulin, as the two factors together had a greater impact than either individually. The additive effect may be explained mechanistically by greater p-Akt/Akt in muscle incubated with both, as Akt phosphorylation is a major step in insulin signaling (Saltiel and Kahn, 2001). However, no such synergistic effect was observed for glucose uptake rates despite enhanced Akt phosphorylation. Unlike β2, β1 stimulation did not appear to affect basal or insulin-stimulated glucose uptake or oxidation, possibly due to the relatively low expression of the β1 isomer compared to β2 (Kim et al., 1991).

Acute stimulation with the pro-inflammatory cytokines TNFα and IL-6 increased glucose oxidation independently of insulin and, in fact, appeared to antagonize insulin when incubated together, as muscle co-incubated with insulin and either cytokine had lower Akt phosphorylation than muscle incubated with insulin alone. Pro-inflammatory cytokines have long been linked to insulin resistance (Heliövaara et al., 2005; Lazar, 2005) which is evident by the decrease in insulin-stimulated Akt phosphorylation in our study. Yet, our results indicate that cytokines may also initially be assuming insulin's role of stimulating glucose oxidation via a non-Akt mechanism, as previously postulated in humans (Saini et al., 2014).

IMPLICATIONS

Metabolic changes that result from acute stress factors may initially be more beneficial than previously understood and may include compensation for reduced insulin action. Conversely, other stress factors like adrenergic stimulation appear to boost insulin's metabolic actions. Together, our findings show that components of the stress response may help to maintain glucose metabolism during the initial onset of stress.

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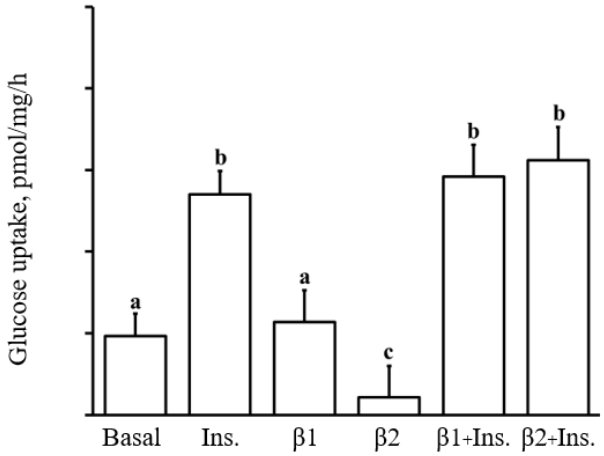

Figure 1. Glucose uptake in primary rat soleus strips (25-45 mg) during a 20-min incubation with the following treatments: none (basal), insulin (5 mU/ml Humulin-R), β1 agonist (10 μM ractopamine HCl), β2 agonist (0.5 μM zilpaterol HCl), β1+insulin, or β2+insulin. (n = 10 rats). ^{a,b,c} means with different superscripts differ ($P < 0.05$).

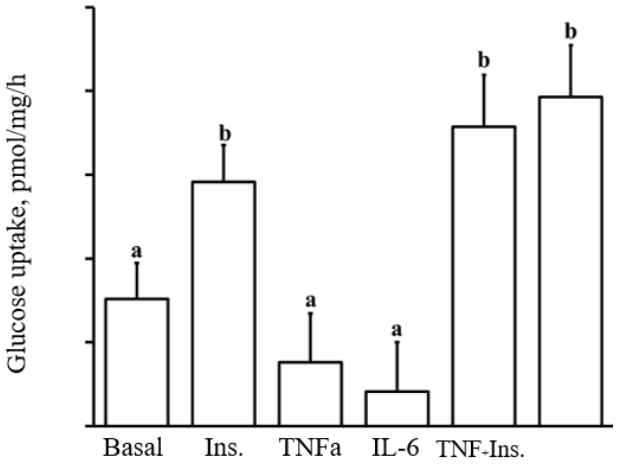

Figure 2. Glucose uptake in primary rat soleus strips (25-45 mg) during a 20-min incubation with the following treatments: none (basal), insulin (5 mU/ml Humulin-R), TNFα (20 ng/ml hTNFα), IL-6 (1 ng/ml rIL-6), TNFα+insulin, and IL-6+insulin. (n = 10 rats). [a,b] means with different superscripts differ ($P < 0.05$).

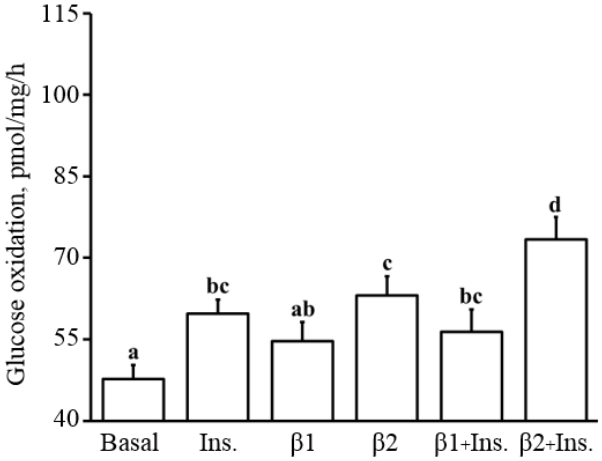

Figure 3. Glucose oxidation in primary rat soleus strips (25-45 mg) during a 2-h incubation with the following treatments: none (basal), insulin (5 mU/ml Humulin-R), β1 agonist (10 μM ractopamine HCl), β2 agonist (0.5 μM zilpaterol HCl), β1+insulin, or β2+insulin. (n = 9 rats). [a,b,c,d] means with different superscripts differ ($P < 0.05$).

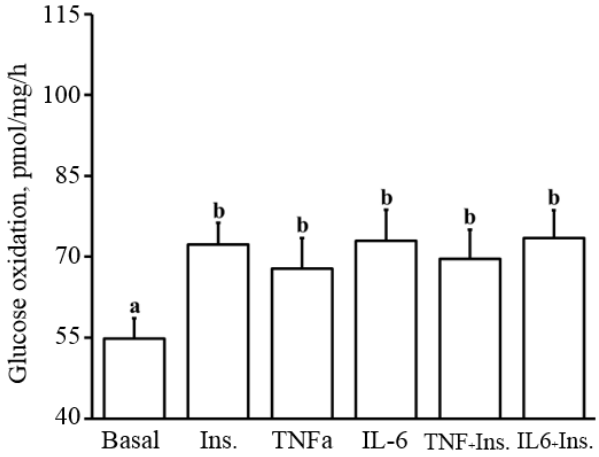

Figure 4. Glucose oxidation in primary rat soleus strips (25-45 mg) during a 2-h incubation with the following treatments: none (basal), insulin (5 mU/ml Humulin-R), TNFα (20 ng/ml hTNFα), IL-6 (1 ng/ml rIL-6), TNFα+insulin, and IL-6+insulin. (n = 9 rats). [a,b] means with different superscripts differ ($P < 0.05$).

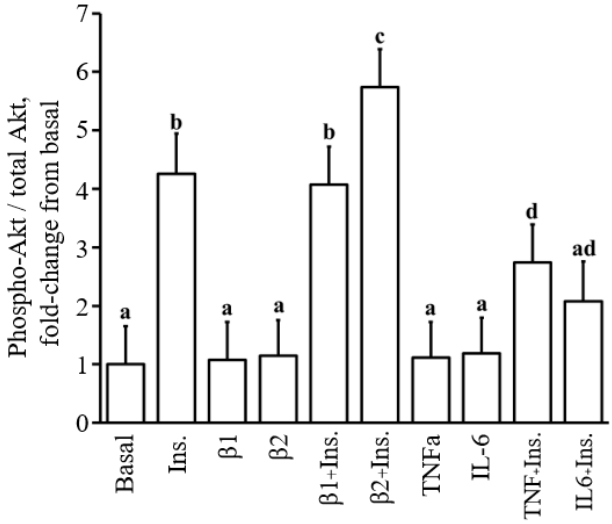

Figure 5. Akt phosphorylation in primary rat soleus strips (25-45 mg) during a 1-h incubation with the following treatments: none (basal), insulin (5 mU/ml Humulin-R), β1 agonist (10 μM ractopamine HCl), β2 agonist (0.5 μM zilpaterol HCl), β1+insulin, β2+insulin, TNFα (20 ng/ml hTNFα), IL-6 (1 ng/ml rIL-6), TNFα+insulin, and IL-6+insulin. (n = 8 rats). [a,b,c,d] means with different superscripts differ ($P < 0.05$).

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Mycobacterium avium subspecies *paratuberculosis* serum lipid profile analysis through Fourier transform ion cyclotron resonance mass spectrometry

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ABSTRACT: *Mycobacterium avium* subspecies *paratuberculosis* (**MAP**) is responsible for Johne's disease (paratuberculosis; **paraTB**) in bovine which elicits serve enteritis in the lower intestinal tract; similar to Crohn's disease in humans. The objective of our study was to observe lipid changes in serum extracts of cattle infected with MAP using ultra-high resolution mass spectrometry. We hypothesized through the use of Fourier transform ion cyclotron resonance mass spectrometry (**FT-ICR MS**) the identification of unique lipid biomarkers induced by MAP infection will be observed. Field samples from cattle infected with MAP (**INF**; n=10) were provided by the National Animal Disease Center (**NADC**). Uninfected serum (**SC2012** and **SC2015**) from cattle with no history of paraTB came from two sources provided by the NADC. Negative controls used to test cross reactivity, with no MAP infection or previous exposure, included serum extracts from cattle challenged with lipopolysaccharide (**LPS**) and cattle infected with *Mycobacterium bovis* (**bTB**). Spectral differences were observed in the INF treatment compared to all other samples. Heteroatom class distribution, in positive ion mode, showed higher relative lipid abundance in the INF treatment in O_5Na_1, O_3, O_9Na_1, $O_{10}Na1$, O_8Na_1, N1, O_7Na_1, and N_1O_1 compounds whereas $N_1O_8P_1$ and N_1O_7P1, were higher abundance in both control treatment groups. In negative-ion mode, O_3 compounds were greater relative abundance in the INF treatment and both control treatments had higher relative abundance of $N_1O_{11}P_1$ and $O_{13}S_1$ compounds. Assigned elemental compositions were searched in the Lipidomic Gateway Databased to identify relative abundance of lipid classes. The majority of compounds classified in the glycerophospholipid, polyketide, or sterol class. Bioinformatics showed forty-five unique compounds ($p \leq 0.05$), twenty-six in positive-ion mode and nineteen in negative-ion mode, purely present in MAP infected cattle; no cross reactivity observed. The shift in heteroatom class distribution provides specific lipids may be present only in paraTB infected cattle, which is confirmed by the compounds identified solely in MAP infected cattle. The use of FT-ICR MS to identify the lipid profile of MAP infected cattle provides an understanding of complex mixtures that can identify unique biomarkers in diseases, primarily with zoonotic properties, to be used as biomarkers to develop innovative diagnostic tools.

Key words: Johne's disease, paratuberculosis, *Mycobacterium avium* subspecies *paratuberculosis*, ultra-high resolution mass spectrometry, lipidomics

INTRODUCTION

Johne's disease primarily affects dairy cattle and may cost producers with Johne's positive herds an economic loss upwards of US $800 per cow (Ott et al., 1999). *Mycobacterium avium* subspecies *paratuberculosis* (**MAP**) is the bacterium responsible for Johne's disease in ruminants, specifically cattle. This pathogenic bacterium is a Gram-positive, facultative anaerobe that is slow growing (Yayo-Ayele et al., 2001). The MAP bacillus can remain viable for up to 11 months in bovine feces and soil and is resistant to many disinfectants (Chiodini et al., 1984). The bacterium leads to thickening of the intestinal wall of cattle which reduces the capability to absorb nutrients resulting in poor performance (Ott et al., 1999). Diagnosis of MAP is often time consuming and expensive, partly due the potential presence other closely related bacteria such as *Mycobacterium bovis* which is responsible for bovine tuberculosis which elicits the same clinical disease as human tuberculosis (Vary et al., 1990). Mass spectrometry could serve as a lipidomic analysis method by providing a broad detection of lipid compounds in serum through a single measurement at a high sensitivity. Fourier transform ion cyclotron resonance mass spectrometry (**FT-ICR MS**) utilizes ultrahigh resolving power and mass accuracy to identify thousands of compounds otherwise unknown in a single spectrum from a complex mixture. The use of FT-ICR MS could provide a framework to track the lipid profile of animals infected with MAP. The objective of our study was to discover lipid changes in serum extracts of MAP infected cattle. We hypothesized that the use of FT-ICR MS on serum extracted form cattle infected with MAP will provide a unique identification of lipid biomarkers when compared to an uninfected control, LPS infected serum, and *M. bovis* infected serum.

MATERIALS AND METHODS

[1]Appreciation is expressed to United States Department of Agriculture-National Animal Disease Center for their assistance with this project.

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Serum Extract Samples

Serum extract samples were obtained according the guidelines and approved animal care protocols implemented by the National Animal Disease Center (**NADC-USDA**, Ames, IA). Field samples from cattle of different ages, sexes, and breeds naturally infected with MAP (**INF**; n=10) were provided by NADC-USDA. Uninfected serum came from two sources from the NADC-USDA; 1) **SC2012** (n=7) were collected in a bovine tuberculosis study from a dairy herd in Minnesota with no history of Johne's disease and 2) **SC2015** (n=10) were provided from cattle with no history of paraTB. Other serum extracts were obtained from cattle challenged with lipopolysaccharide (**LPS**) at New Mexico State University to serve as negative controls. These samples were used to test the robustness of the system using samples from cattle that were infected with a portion of a bacterial cell. Finally, serum provided by the NVSL from cattle infected with *Mycobacterium bovis* resulting in bovine tuberculosis (**bTB**) with no MAP infection or previous exposure was used to test cross reactivity of the bacterium. All serum lipid extracts, except LPS, were sent from NADC-USDA and NVSL-USDA and extracted in Ames, IA. Whole blood samples were centrifuged to harvest serum from all treatments. Then 200 µL of EDTA was added to a 2 mL serum aliquot in 2 mL Eppendorf tubes. For SC2012, 320 µL of internal standard mix, (17:0/17:0) phosphatidylethanolamine (**PE**; Avanti Polar Lipids, Alabaster, AL, CAT#830756P) at 1:1 mM in methanol, was added and mixed thoroughly. In addition to PE 17:0/17:0, SC2015 also contained an internal standard mix that consisted of 4 mM 2-hydroxyoleic acid (OHOA; Avanti Polar Lipids, CAT#830756P) and 0.6 of 5-(palmitoyloxy) octadecanoic acid (PAHSA) (Avanti Polar Lipids, Alabaster, AL, CAT#900410) in 1:1 chloroform: methanol. For MAP infected serum 0.43 mM PE was added as the internal standard. The addition of 1 mL of methyl tert-butyl ether (**MTBE**) was incorporated in the sample and vortexed for 1 hr at 20°C. Once vortexed, 250 µL of MS grade water was added and vortexed thoroughly then the sample was centrifuged at 4000 rpm for 1 min before 800 µL of upper MTBE phase was transferred into a vial and stored at - 20°C until shipped to New Mexico State University Analytical Chemistry Laboratory. Lipopolysaccharide serum was extracted at conducted at New Mexico State University, rumen microbiology laboratory using the same extraction method described above with the addition of internal calibrants in the ionization spray and not at the time of serum lipid extraction.

ESI FT-ICR Mass Spectrometry

High resolution FT-ICR MS was performed with a hybrid linear ion trap 7 T FT-ICR mass spectrometer (LTQ FT, Thermo Fisher, San Jose, CA) equipped with an Advion Triversa Nanomate (Advion Biosystems, Inc.). Mass resolving power was m/Δm50% = 400,000 at mass-to-charge (**m/z**) 400 and the time-domain transient length was 3 s (Sudasinghe et al., 2104). A total of 200 time-domain transients were co-added for each sample prior to fast Fourier transformation and frequency to mass-to-charge ratio conversion. Serum MAP lipid extracts were analyzed through direct infusion electrospray ionization or by the Advion Triversa Nanomate. For positive-ion mode analysis, INF extracts were diluted 20-fold and SC2012 lipid extractions were diluted 10-fold in an electrospray ionization (**ESI**) solution of 1:2:4 chloroform: methanol: 2-propanol containing 0.1% formic acid. Serum control 2015 (SC2105) and LPS serum extracts were diluted 10-fold in an ESI solution of 2:1 methanol: HP mix containing 0.1% formic acid. The addition of formic acid facilitates protonation in positive-ion mode. The HP mix used in the SC2015 serum extracts provides internal calibration to the samples serving as validation targets when analyzed by bioinformatics. For negative-ion mode, INF and SC2012 lipid extracts were diluted 10-fold in an ESI solution of 1:2:4 chloroform: methanol:2-propanol containing 0.1% ammonium hydroxide. Serum control 2015 and LPS serum extracts were diluted 10-fold in an ESI solution of 2:1 methanol: HP mix containing 0.1% ammonium hydroxide. The addition of ammonium hydroxide facilitates deprotonation in negative-ion mode.

FT-ICR mass spectra were internally calibrated and peak lists were generated to include signals with signal-to-noise (S/N) >10. Elemental compositions were assigned using PetroOrg software (Florida State University, Tallahassee, FL) with interations of molecular constraints $C_cH_hN_3O_{25}S_3P_1Na_1$. Assigned elemental compositions were grouped into compound classes with the same heteroatom content to perform class analysis. The Lipidomics Gateway database (**LIPID MAPS**) was used to assign lipid classifications to elemental formula assignments in both control treatments and MAP infected serum.

Bioinformatics

Bioinformatic analysis was performed by the Department of Computer Science at NMSU using statistical and data mining techniques. The t-test was used on the entire lipid set – all lipids found in INF, SC2012, and SC2015. This was repeated with three different alternative hypotheses containing a batch that is biased towards paraTB cattle, a second batch that is biased towards control cattle, and a third batch that is not biased towards any treatment to cover the sample space and ensure no important compound is missing. All lipids with $P \leq 0.05$ were copied and placed into two different sample sets. The first sample set P-value was adjusted using the Benjamini-Hotchberg method, which controls the family wise false discovery rate. Post adjustment, all $P \leq 0.05$ lipids were grouped into lipid set 1. The second sample set is used to generate a preliminary boxplot and placed in the Principal Component Analysis (**PCA**) module to eliminate outliers. After PCA is preformed, these lipids were used to construct a training data set that was given to a random forest classifier (**RFC**) which reports the importance of factors and in our case lipids, to achieve the reported accuracy. All lipids that helped achieve 100% accuracy are

reported. We selected lipids that scored a non-zero importance value to form a data set named lipid set 2.

Lipid set 1 and 2 are merged together to obtain lipid set 3. Each individual lipid was fed into RFC to analyze how well they classify for paraTB on their own. Lipids that have non-zero values for all INF lipid samples or all control lipid samples would report an accuracy of 100% since they represent the sample space that is linearly separable. Biased towards paraTB showed forty-five lipids, both in protonated and deprotonated state, with reported 100% accuracy. These lipids are collected and grouped into a final sample set named "finstate."

RESULTS AND DISCUSSION

When comparing the spectra of INF, SC2012, SC2015, and LPS infected cattle there is a difference seen visually in both ionization modes. Positive-ion mode showed paraTB had the largest amount of peaks found, at ten sigma, when compared to both control spectra and LPS. Paratuberculosis spectra also shows a group of lipids, represented by peaks, not found in either control groups or LPS in the 300-400 m/z range, which primarily classify in the mono-, di-, and triacylglycerols. Both control spectra have a higher abundance of lipids in the 700-900 m/z range when compared to INF and LPS, identifying with complex compounds, such as glycerophospholipids. The LPS spectrum shows a high abundance of lipids throughout the entire spectra which is not observed in either control or INF treatments (Figure 1). Negative-ion mode showed a vast amount of peaks found in the paraTB and both control spectra when compared to LPS but a difference in spectra is also observed (Figure 2). Lipids are only seen in the 500-700 m/z range in INF compared to both control and LPS. The INF spectra differs in the lipid fraction, in both ionization modes, by variation in peak distribution and peak height, represented by relative abundance (%), when compared to SC2015, SC2012, and LPS. This shows different lipid classifications are present within each treatment.

The majority of classifications were in lipid classes: Glycerophosphocholines (**PC**), Glycerophosphoethanolamines (**PE**), Glycerophosphoserines (**PS**), Phosphatidic acids (**PA**), Diacylglycerols (**DAG**), Triacylglycerols (**TAG**), Phosphosphingolipids (**SM**), Oxidized Triacylglycerols (**OxTAG**), Oxidized Glycerophospholipids (**OxGP**), Prenol Lipids (**PR**), Sphingoid bases (**SP**), Polyketides (**PK**), Glycerophosphoinositols (**PI**), Glycerophosphoglycerols (**PG**), Sterols, and Fatty Acyls (Figure 3-4). Mycobacteria do not produce PC or SP which is reflected in the lipid classification graph where animals infected with MAP have a lower relative abundance of both lipid classes when compared to control groups (Layre et al., 2014). Potentially showing when whole blood is pulled and the serum lipid is extracted portions of the bacterial cell wall are present, along with the host (cattle) cell walls, accounting for the lower abundance of PC and SP lipid classes in the INF treatment. Glycerphosphocholines are a major component in biological membranes that is the principal phospholipid in humans and absent in the membrane of most bacteria's exoplasmic region (Berg et al., 2002). In the 600-700 m/z range a higher abundance of signals are observed. Sphingoid bases protect the surface of the cell from harmful environments, which may be a result of previous bacterial exposure in the SC2015, which are the highest in relative abundance compared to SC2012 and INF treatments. Three heteroatom classes $N_1O_7P_1$, $N_1O_8P_1$, and $N_2O_6P_1$ were primarily identified and most of the compounds within each, roughly 90%, matched lipid classes with the glycerophospholipid (GP) family. The lipids identified in the serum give insight to biological functions associated with INF, SC2015+SC2012, and LPS.

Positive-Ion Mode

Heteroatom class distribution demonstrates all control and paraTB infected cattle have seventeen common classes. Of that, relative abundance differences are seen in ten heteroatom classes (Figure 5). The top three most abundant heteroatom classes where INF was significantly different from the controls classified in the following lipid classes: O_5Na_1 compounds assigned to Fatty Acyls, PR, PK, and Sterols; O_9Na_1 classified in Fatty Acyls, TAG, Sterols, PR, and PK; lastly, O_3 identified with PK and Fatty Acyls. Naturally occurring Fatty Acyls commonly have an even number carbon chain and cells use Fatty Acyls to yield large quantities of ATP. Polyketides are secondary metabolites and also utilized in virulent mycobacteria on the surface of cell membranes to interact with host cells, which may explain why the INF treatment group had a higher relative abundance of PK compounds, if the bacterial cell wall is present in the lipid extraction sample (Jain and Cox, 2005; Figure 3). Bacteria lack sterols, which have a basic cholesterol precursor structure to fat soluble vitamins and steroid hormones (Saenz et al., 2012). Interestingly, sterols were in the highest abundance in MAP infected cattle, warranting further investigation.

In both control groups, N_1O_8P1 and $N_1O_7P_1$, were higher relative abundance and each classified in the lipid class glycerophospholipid which incorporates PA, PC, PE, PG, and PI that are all important lipids found in cell membranes. Glycerophosphoethanolamines, PE, compose 25% of all phospholipids in cell membranes and is the only principal phospholipid in bacteria (Berg et al., 2002). The SC2015 treatment showed the highest abundance for PE lipid compounds. This treatment was comprised of animals consisting of varying age, sex, breed, and location where previous exposure to bacteria is unknown. As a result, host serum samples may contain compounds, via adaptive immune compounds or lipids from previous or current exposure from varying bacterial infections, which classify as PE's giving rise to why the SC2015 have the highest relative abundance within this lipid classification.

For bioinformatics analysis, S/N ratio normalized to the internal standard (17:0/17:0) PE were provided and alignments were applied to isolate specific compounds present in INF that are absent in all control

samples. Overall there were twenty-six compounds that were present purely in INF and not found in either control treatment, LPS, or bTB. Thirteen of the compounds were assigned to an elemental composition where three classified as, $C_CH_HO_5$; $C_CH_HO_4Na_1$; $C_CH_HO_4Na_1$, in LIPID MAPS to known lipids in the PK or monoacylglycerols subclass. Two compounds, $C_CH_HN_1O_4$ and $C_CH_HO_{10}N_1$, were not in either SC2012 or SC2015 but both found in LPS and $C_CH_HN_1O_4$ was also found in bTB; these compounds may be potential immune response compounds.

Negative-Ion Mode

Heteroatom class distribution designates thirteen common classes were identified in INF and both SC2012 and SC2015. Among these classes, the O_x series was prevalent however $O_{13}S_1$, $O_{20}P_1$, $N_1O_{10}P_1$, $N_1O_{11}P_1$, $N_1O_{10}P_1Cl1$, and N_2O_{15} were also identified (Figure 6). Only three were different: O_2 showed to have a higher relative abundance in paraTB, whereas $O_{13}S_1$ and $N_1O_{11}P_1$ classes were higher in both SC2012 and SC2015. In the O_2 class, the majority of compounds identified assigned to branched fatty acids in the Fatty Acyls group however some compounds did classify as PK, PR, and Sterols. The $O_{13}S_1$ heteroatom class did not assign to any known lipid classifications through the Lipidomic Gateway library. However, nearly all compounds identified as $N_1O_{11}P_1$ classified as a glycerophospholipid with the majority in the PC class, however PE, PS, and PG were also hits in the library. Glycerophosphocholines, PC, are a major component in eukaryotic cell membranes and are absent in the membranes of bacteria's endoplasmic region which is reflected in a lower abundance in INF extracts compared to a higher abundance in controls (Rock et al., 2002). Glycerophosphoinositols, PI, have recently been shown to act as modulators for T-cell signaling and responses involved with the adaptive immune system (Corda et al., 2009). In addition, a small set of compounds in the $N_1O_{11}P_1$ class were recognized as sterols.

Bioinformatics showed a total of nineteen unique compounds found purely in INF where seventeen were assigned to an elemental composition and five matched to a specific lipid identification in LIPID MAPS. This included two $C_CH_HO_4$ compounds and $C_CH_HO_5$ classifying as PK, $C_CH_HO_2$ classifying as a C_{21} steroid (gluco/mineralocorticoids, progesterones), and $C_CH_HO_2$ as a Fatty Acyl or androgren species. Two compounds were found in INF, LPS, and bTB serum extracts but not either of the control treatment groups. These consist of $C_CH_HO_8$ and $C_CH_HO_2$, which the latter classified as either a Fatty Acyl or Sterol.

The results of our study show that the lipid class profile abundance in cattle infected with MAP is detectable through the FT-ICR MS and that a spectral and heteroatom class difference is present among MAP infected cattle compared to uninfected, LPS infected, and bTB infected cattle. The use of ultra-high resolution mass spectrometry could provide a gateway to monitor the lipid changes in serum of infected cattle to potentially create a rapid field test to aid in the eradication of the disease.

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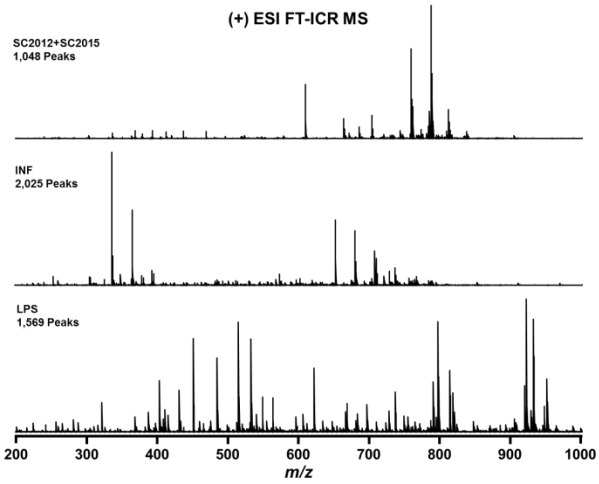

Figure 1. Positive-ion mode ESI broadband FT-ICR mass spectra showed a spectral difference between all controls, INF, and LPS infected cattle serum lipid extracts.

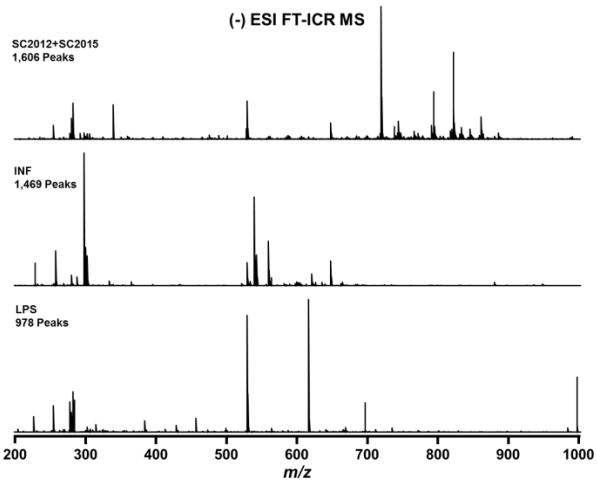

Figure 2. Negative-ion mode ESI broadband FT-ICR mass spectra showed a spectral difference between all controls, INF, and LPS infected cattle serum lipid extracts.

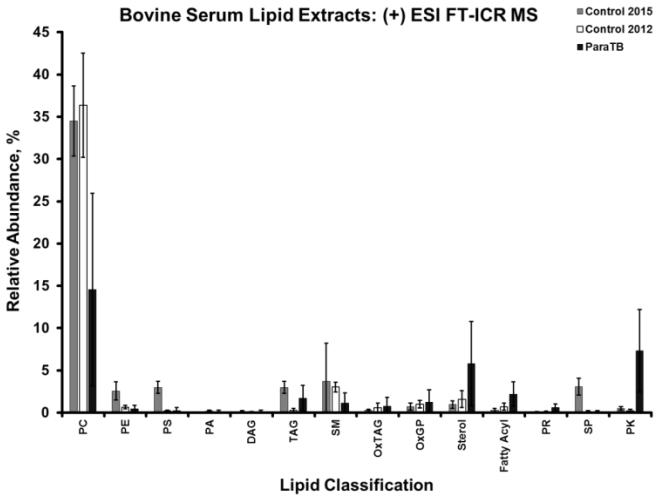

Figure 3. Lipid classification bovine serum lipid extracts:(+) ESI FT-ICR MS. Lipid classification assignment and relative abundance found in paraTB, control 2012, and control 2015 serum lipid extracts.

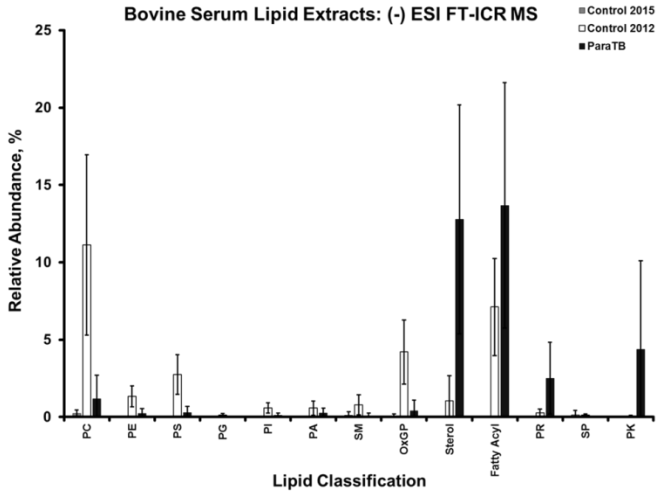

Figure 4. Lipid classification bovine serum lipid extracts: (-) ESI FT-ICR MS. Lipid classification assignment and relative abundance found in paraTB, control 2102, and control 2015 serum lipid extracts.

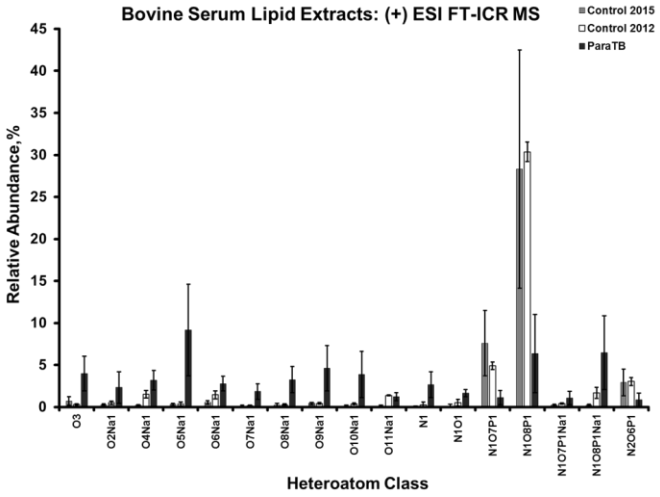

Figure 5. Heteroatom class bovine serum lipid extracts: (+) ESI FT-ICR MS. Positive-ion mode ESI heteroatom class assignment and relative abundance found in paraTB, control 2102, and control 2015 serum lipid extracts.

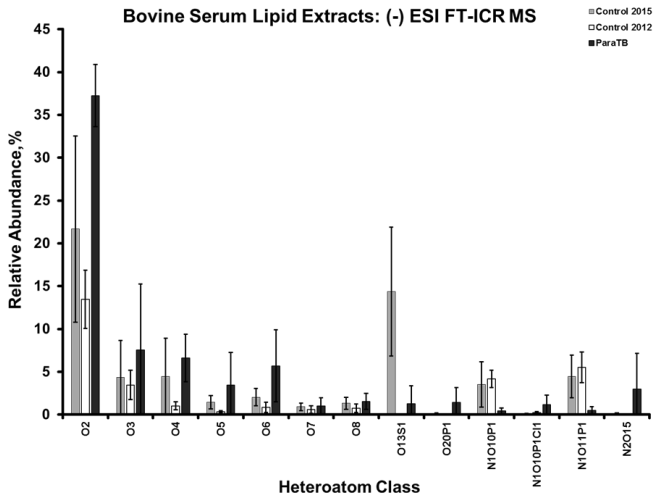

Figure 6. Heteroatom class bovine serum lipid extracts: (-) ESI FT-ICR MS. Negative-ion mode ESI heteroatom class assignment and relative abundance found in paraTB, control 2102, and control 2015 serum lipid extracts.

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Relationship between current temperament measures and physiological responses to handling of feedlot cattle

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ABSTRACT: Interest in beef cattle temperament has increased due to growing consumer awareness of animal welfare. Researchers have defined temperament as behavioral responses to a perceived stressor. Subjective chute scoring has been used by many researchers for temperament, however, the subjectivity and associated variability among observers has been questioned. The most practical objective method of assessing temperament is exit velocity. Corresponding chute side measures to physiological markers is important. Faster exit velocities have been related to both increased cortisol and increased plasma lactate. The objectives of this study were to compare temperament differences between feedlot steers and heifers and to confirm chute side measures relationship to physiological responses to stress. Body temperature, serum and plasma lactate, serum glucose, salivary and serum cortisol concentrations were measured on mixed breed and sex feedlot cattle (n = 197). Fast, medium, and slow classifications were developed from exit velocities. Plasma lactate was significantly different between all classes. Sex had a significant effect on exit velocity and physiological measures. Heifers had higher exit velocities (P = 0.003), plasma lactate concentrations (P = 0.03), and cortisol concentrations (P = 0.001). Simple correlations among these variables indicated body temperature (heifers r = 0.44, P < 0.0001; steers 0.45 P < .0001), plasma lactate (heifers r = 0.52 P < 0.0001; steers r = 0.63 P < 0.0001), serum lactate (heifers r = 0.53 P < 0.001; steers r = 0.59 P < 0.001) and glucose (heifers r = 0.54 P < 0.001; steers r = 0.32 P <0.003) were all correlated to exit velocity in both steers and heifers. Cortisol measures were not correlated to exit velocity in steers but were in heifers. Linear models constructed and evaluated using Akaike information criterion indicated that plasma lactate in combination with body temperature were strong candidates to predict exit velocity. Using the discriminate function analysis, the model categorized fast and slow classifications 69.23% and 61.54% respectively, indicating that in combination with exit velocity simple objective chute side measures of body temperature and plasma lactate can potentially increase accuracy of temperament identification.

Key words: Cortisol, Exit Velocity, Lactate, Temperament

INTRODUCTION

Renewed interest in the temperament of beef cattle has occurred in response to concerns for animal welfare by consumers within the United States (Lyles and Calvo-Lorenzo, 2014). Additionally, temperament has a direct impact on feedlot performance, carcass quality, and meat quality (Voisinet et al., 1997a; Voisinet et al., 1997b; Ferguson et al., 2006; Cafe et al., 2011; Boles et al., 2015).Temperament has been defined as how an individual reacts to a novel or challenging situation (Fordyce et al., 1988; Grandin, 1998; Curley et al., 2006; Ferguson et al., 2006; Cafe et al., 2011). Temperament of beef cattle has been evaluated using a variety of subjective and objective methods that evaluates the animal's response to human interaction. Currently, exit velocity, defined as the speed at which an animal exits a chute, is recognized as the most practical objective measure for assessing temperament (Cafe et al., 2011). Subjective chute scoring systems have also been used by many researchers (Fordyce et al., 1988; Voisinet et al., 1997b; Fell et al., 1999; Francisco et al., 2012). Due to the subjectivity and associated variability among researchers, chute scores have been questioned for repeatability and consistency.

Temperament influences the amplitude of response from the hypothalamic-pituitary-adrenal axis (HPA) to a stressor. The perception of a stress initiates a cascade of endocrine reactions, in an attempt to maintain homeostasis (Curley et al., 2008). This concomitant response of cortisol, epinephrine, and associated increase in heart rate, body temperature, and metabolic processes could provide biomarkers that could aid in defining an individual animal's temperament (Tsigos and Chrousos, 2002, Burdick et al., 2011b). In studies investigating biomarkers as potential indicator of stress, blood lactate was found to be significantly correlated with chute score and exit velocity (Boles et al., 2015). Moreover, Curley et al. (2006) reported that cortisol, measured in blood from Brahman bulls was correlated to exit velocity. The purpose of this study was to compare chute scores and exit velocities to physiological responses of, body temperature, metabolites and hormones, to find a biomarker which could improve objective temperament classifications.

MATERIALS AND METHODS

Research was conducted under animal care protocol (MSU 2014-AA09) approved by the Montana State University Agricultural Animal Care and Use committee.

One-hundred and ninety-seven (n = 197) feedlot cattle were sampled from a commercial, certified Beef Quality Assurance feedlot in Chappell Nebraska. Animals were fed a standard concentrate feedlot diet, consisting of 94% concentrate feed composed of rolled corn, beet pulp, dried distillers grains, protein supplement, corn silage and ground hay: Diets fed to mixed sex pens included melengestrol acetate (MGA), 0.5 mg/day, to suppress estrous in females. A Polaris timer system (Farmtek Inc., Wylie Texas) was used to measure exit velocity with photo-transmitters placed 1.83 and 3.65 meters in front of chute. Chute scores were assigned by the same individual for each sampling and were based on the chute scoring system recommended by Beef Improvement Federation.

Body temperature was measured with a Jorgensen Laboratories Digital Thermometer fitted with a rectal probe (Jorgensen Laboratories, Loveland CO, USA). Blood samples were drawn from the jugular vein using Vacuette® needles (Greiner Bio-One, Kremsmünster, Austria) into one of two different Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes NJ, USA) while the animals were restrained in a hydraulic squeeze chute (Moly Manufacturing, Lorraine KS, USA). Silicone-coated tubes were used when measuring lactate and cortisol, and sodium fluoride potassium oxalate-coated tubes were used when glucose was to be measured. Tubes were stored on ice during collection and were centrifuged at 2,700 rpm for 30 minutes following which serum was decanted. Two salivary samples were collected from each animal using Salivette tubes (Sarstedt AG, Nümbrecht, Germany). An Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit from Salimetrics® (Salimetrics, State College PA, USA) was used to analyze saliva for cortisol levels. Lactate Assay Kit II (Biovision Inc, Milpitas CA, USA) micro-plate assay procedure was used for serum lactate concentrations. Plasma blood lactate was measured chute side in < 2 µL of blood from jugular venipuncture using a Lactate Pro® meter (Akray Inc. Minami-ku, Kyoto Japan). Serum glucose concentrations were determined using Infinity™ Glucose (lot # 241531) (Thermo Scientfic, Waltham NC, USA) micro-plate assay kit.

The General Linear Model and Least Square Means procedure of SAS (SAS 9.4, 2014) was used to analyze differences and calculate means between temperament classifications and physiological measures, where the class variable was exit velocity classification and sex. Exit velocity classification was based on individual animals exit velocity sorted fast to slow with the fastest one-third being classified as fast, middle one third as medium and the last one-third as slow. The dependent variables were chute score, body temperature, plasma lactate, individual exit velocity, glucose, serum lactate, salivary and serum cortisol. Because of the significant ($P \leq 0.04$) sex effect, Pearson product-moment correlations were calculated by sex. Linear models were analyzed (R-Studio version 2.15.1) where exit velocity was compared to the variables and combination of variables of plasma lactate, body temperature, glucose, salivary and serum cortisol to determine which measure or measures could possibly be used as an objective temperament measure similar to exit velocity. An Akaike information criterion (AIC) was used to analyze the quality of the models. The lowest AIC values are reported here for steers and heifers indicating the best candidate linear models to predict exit velocity. A discriminate function analysis was used to analyze (SAS 9.4, 2014) the top candidate model from the AIC. All data were considered significant when the P-value was less than 0.05.

RESULTS AND DISCUSSION

The purpose of this study was to compare chute scores and exit velocities to physiological responses of body temperature, metabolites and hormones, to potentially find a biomarker which could improve objective temperament classifications.

Table 1. Least square means for body weight (WT), chute scores (CS), body temperature (TEMP), blood lactate as measured by the handheld meter (PLAC), exit velocity (EV), serum glucose (GLUC), serum lactate (SLAC), and salivary cortisol (SCORT) or serum cortisol (BCORT) classed by sex for feedlot steers and heifers.

Item	STEERS	SEM	HEIFERS	SEM	*P*-Value
n	87		109		
WT (kg)	426.85	9.04	425.33	7.84	n.s.
CS[1]	2.94	2.9	3.24	3.2	***
EV (m/s)	2.24	0.14	2.80	0.12	**
TEMP (°C)	39.78	0.05	39.93	0.05	*
GLUC (mg/dL)	104.72	3.74	112.04	3.30	n.s.
PLAC[2] (mM)	3.45	0.31	4.35	0.26	*
SLAC (mM)	5.43	0.44	6.05	0.39	n.s.
SCORT (µg/dL)	0.18	0.02	0.26	0.01	***
BCORT (µg/dL)	1.64	0.11	2.13	0.09	***

Significance = $*P < 0.05$ $**P \leq 0.01$, $***P \leq 0.001$, **** $P \leq 0.0001$
Values are Least Square Means. Significantly different $P < 0.05$.
[1]Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty(Wild) 5= Aggressive, 6 = Very Aggressive
[2]Lactate Pro® Meter

Sex had a significant effect on chute score, exit velocity, body temperature, plasma lactate and cortisol measures. Data indicated heifers were more excitable than steers. Comparable results for sex differences in exit velocities have been reported in Hoppe et al. (2010). Similar temperament differences have also been reported in heifers and steers by Voisinet et al. (1997b). Furthermore, body temperatures were significantly higher in heifers ($P < 0.05$) compared to steers. Burdick et al. (2011b) evaluated body temperatures of bulls classified as calm, intermediate, and excitable, prior to and during a lipopolysaccharide (LPS) challenge. The peak in serum epinephrine in calm bulls coincided with a rise in body temperature suggesting that it was a strong indicator of a stress response. Our data in combination with Burdick et al. (2011b) supports body temperature rises as an indicator of stress due to handling. Elevated body temperatures

reported in this study could be due to handling but also reflects temperament differences.

Lastly, salivary and serum cortisol concentrations were significantly ($P \leq 0.001$) higher in heifers than steers. Cooke (2014) reported mean circulating plasma concentrations of cortisol in heifers described as having adequate temperament to be lower than in heifers described as having excitable temperaments.

In summary, animals with faster exit velocities or classified as fast, had a higher physiological response to handling than did animals classed as medium or slow. Furthermore, plasma lactate also sorted animals into three distinct classifications making it a candidate for an objective measure of temperament similar to exit velocity.

Selected Pearson correlations are reported for heifers and steers (Table 3). Body temperature was moderately correlated to blood lactate in both heifers and steers however the correlation was stronger in steers ($r = 0.497$, $P < 0.0001$) than in heifers ($r = 0.398$, $P < 0.0001$). Additionally, body temperature was correlated to metabolites and exit velocity and moderately correlated to glucose, plasma and serum lactate, and salivary cortisol (Table 3). Interestingly, heifers had the highest correlation between body temperature and salivary cortisol concentration ($r = 0.567$, $P < 0.0001$). Gruber (2010) also found a positive correlation between body temperature and serum lactate concentrations ($r = 0.14$). Additionally, they reported a positive correlation between body temperature and serum cortisol ($r = 0.33$). Plasma lactate was also significantly correlated to metabolites and hormone measures. Importantly, plasma lactate as measured by the Lactate Pro® meter was highly correlated to serum lactate in both heifers and steers. This agrees with the validation study of Burfeind and Heuwieser (2012) who reported a strong correlation ($r = 0.98$) between measures from the blood lactate meter and serum lactate measured in a laboratory.

The data presented indicated that as exit velocity increased both lactate measures increased. These results agree with Coombes et al. (2014) who reported that as flight speed increased plasma and muscle lactate increased. Moreover, lactate was moderate to highly correlated ($r = 0.64$) to systemic glucose concentrations. These findings combined with the finding from Gruber (2010) and Coombes et al. (2014) demonstrated that excitable animals mobilized glucose through glycogenolysis due to increased energy demand in response to stress in the muscle, resulting in elevated lactate and glucose being transported into the blood.

Exit velocity was not correlated to serum or salivary cortisol concentrations in steers but in heifers there was a positive correlation to both cortisol measures. The strongest correlations with exit velocity were body temperature, plasma and serum lactate, and serum glucose.

Due to the differences found between chute side measures and exit velocity in steers and heifers the models were classed by sex. The AIC data is presented in Table 4. In steers, the combination of plasma lactate and rectal temperature had the strongest AIC weight and therefore represented the best fit model to predict exit velocity. However, in heifers, the prediction using plasma lactate and rectal temperature did not have the same strength as in steers.

The discriminate function analysis (Table 5) of the top candidate model of plasma lactate and body temperature was effective at placing animals correctly in fast and slow classifications 69.23% and 61.54% respectively.

CONCLUSION

Temperament has a direct impact on efficiency and perception of beef cattle production in the United States. This study aimed to identify the relationship of chute side objective measures to physiological to replace or augment exit velocity as a predictor of an animal temperament. Steers and heifers react differently to handling stress as indicated by the significant differences in chute side measures, physiological measures, and exit velocity. Plasma lactate was significantly related to exit velocity and when exit velocity was used as a classification, plasma lactate concentration was also significantly different between the three classes. Furthermore, AIC indicated plasma lactate in conjunction with body temperature was the strongest candidate for predicting exit velocity. The discriminate function analysis indicated plasma lactate and rectal temperature have the potential to become strong objective measures to augment exit velocity to predict an animal's temperament.

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Table 2. Classification of animals by exit velocity for body weight (WT), chute scores (CS), body temperature (TEMP), blood lactate as measured by the handheld meter (PLAC), exit velocity (EV), serum glucose (GLUC), serum lactate (SLAC), and salivary (SCORT) or serum (BCORT) cortisol.

Item	Exit velocity class				
	FAST[1]	MEDIUM	SLOW	SEM	*P*-value[2]
n = 197					
WT (kg)	452.8[a]	402.9[b]	374.7[c]	10.8	****
CS[3]	3.4[a]	3.1[b]	2.8[c]	0.07	****
TEMP (ºC)	40.15[a]	39.78[b]	39.64[b]	0.06	****
EV (m/s)	4.10[a]	2.56[b]	1.06[c]	0.07	****
GLUC (mg/dL)	129.68[a]	101.32[b]	94.63[b]	4.3	****
PLAC[4] (mM)	6.4[a]	3.2[b]	2.4[c]	0.3	****
SLAC (mM)	9.28[a]	4.76[b]	3.86[b]	0.48	****
SCORT (µg/dL)	0.27[a]	0.21[b]	0.17[b]	0.02	****
BCORT (µg/dL)	2.25[a]	1.74[b]	1.69[b]	0.14	**

[1]Exit velocities were separated by thirds with fastest exit velocities being classified as fast, slowest exit velocities as slow and the middle one-third classed as medium.
[2]Significance = **P* < 0.05 ***P* ≤ 0.01, ****P* ≤0.001, **** *P* ≤ 0.0001
[a,b,c] Means within a row that have a different superscript letter differ (*P* < 0.05)
[3]Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty (Wild) 5= Aggressive, 6 = Very Aggressive.
[4]Lactate Pro® Meter

Table 4. AIC values for chute side measures: plasma lactate meter[1] (PLAC), body temperature (TEMP)[2], to predict exit velocity (EXIT) for steers and heifers

	AICc	Δ AICc	AICcWt	Cum.WT
	Steers			
BLM + TEMP	187.30	0.00	0.84	0.84
BLM	190.64	3.34	0.16	1.00
TEMP	200.26	12.95	0.00	1.00
Null EXIT	212.93	25.63	0.00	1.00
	Heifers			
BLM + TEMP	243.20	0.00	0.65	0.65
BLM	244.43	1.23	0.35	1.00
TEMP	260.37	17.17	0.00	1.00
Null EXIT	272.70	29.50	0.00	1.00

[1]Plasma lactate was measured using a Lactate Pro® meter
[2]Body temperature was measured using a veterinary digital thermometer fitted with a rectal probe.

Table 5. Discriminate function analysis for exit classifications[1] using chute side objective measures of plasma lactate[2] and body temperature[3]

Class	Fast	Medium	Slow	Total
Fast	69.23 %	29.23%	1.54%	100%
n =	45	19	1	65
Medium	42.62%	39.34%	18.03%	100
n =	26	24	11	61
Slow	10.77%	27.69%	61.54%	100
n =	7	18	40	65
Total	40.84%	31.94%	27.23%	100%
n =	78	61	52	191
Priors	0.333	0.333	0.333	

[1]Exit velocity classifications were derived by sorting exit velocities highest to lowest and splitting into thirds, first third being fast, second third being medium, and last third being slow.
[2]Plasma lactate was measured using a Lactate Pro® meter.
[3]Body temperature was measured using a veterinary digital thermometer fitted with a rectal probe.

Table 3 Pearson correlation coefficients among body temperature (TEMP), blood lactate as measured by the handheld meter (PLAC), exit velocity (EV), serum lactate (SLAC), serum glucose (GLUC), cortisol (SCORT) or serum (BCORT) salivary for steers and heifers

	STEERS			HEIFERS		
	EV	PLAC	TEMP	EV	PLAC	TEMP
PLAC[1]	0.631 ****	1	0.498 ****	0.529 ****	1	0.398 ****
SLAC	0.591 ****	0.781 ****	0.477 ****	0.534 ****	0.828 ****	0.387 ****
SCORT	0.162	0.127	0.445 ****	0.362 ****	0.375 ****	0.568 ****
BCORT	0.159	0.344 **	0.445 ****	0.218 *	0.330 ***	0.417 ****
GLUC	0.322 ***	0.517 ***	0.540 ****	0.537 ****	0.644 ****	0.419 ****

Significance = **P* < 0.05 ***P* ≤ 0.01, ****P* ≤ 0.001, **** *P* ≤ 0.0001
[1] Lactate Pro ® Meter

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Influence of sampling location and pregnancy on composition of the microbiome associated with the reproductive tract of the ewe

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ABSTRACT: The objective of this study was to investigate the microbiome of the vagina, uterus, and embryo, determine effects of pregnancy status and a maternal pregnancy recognition antagonist treatment. We hypothesized location, pregnancy status, and maternal pregnancy recognition antagonist treatment would result in significant differences in bacterial microbiome in the reproductive tract in sheep. Mini osmotic pumps were placed surgically into the uterus and loaded with control (PBS, n=9) or treatment (AMD3100, n=7). AMD3100 is an antagonist for maternal pregnancy recognition. Samples were collected for microbiome analysis from the vagina, uterus, and embryo. AMD3100 and PBS had no effect on microbiome composition (P > 0.98). Sampling location had the greatest effect on bacterial population (P > 0.01). *Firmicutes*, *Proteobacteria*, and *Actionbacteria* were the most predominant phyla ($P < 0.01$) present in the vagina. While *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were present in the uterus ($P < 0.01$). Genus of bacteria present in the uterus and vagina supported the phylum data. *Corynebacterium* was more prevalent than *Finegoldia* in the vagina ($P < 0.01$), while the prevailing genus in the uterus was *Bradyrhizobium* ($P < 0.01$). Pregnancy status of ewes did not differ by phylum however, the genus *Finegoldia* was greatest in nonpregnant ewes ($P < 0.01$). Treatment effects were not observed on embryo microbiome phylum ($P < 0.90$) or genus ($P < 0.88$). Results show further research is needed to understand the relationship between the reproductive tract microbiome and ewe fertility.

Key words: metagenome, sheep, uterus, vagina

INTRODUCTION

In 2007 the Human Microbiome Project (HMP) was established by the National Institutes of Health. The HMP has characterized microbial populations of skin, gut, mouth, and vagina (Grice and Segre, 2012). The human vaginal microbiome has been well characterized and *Lactobacillus* is the dominant bacterium present (Sirota et al., 2014; Walther-António et al., 2014). *Lactobacilli* are considered important to vaginal homeostasis by helping to maintain a vaginal pH below 4.5 which helps to inhibit vaginal pathogens (O'Halan et al., 2013). When the *Lactobacillus* community is decreased or replaced by other anaerobes, bacterial vaginosis can occur (Mastromario et al., 2014). Bacterial vaginosis has been shown to affect women's fertility by allowing infections to occur in other parts of the reproductive tract in addition to the vagina leading to tubal factor infertility (Mastromario et al., 2014).

Limited and conflicting data are available regarding the bacterial composition of the reproductive tract in livestock species. Swartz et al. (2014) characterized the vaginal microbiota of ewes and cows revealing low levels of *Lactobacilli* compared to levels found in the human vagina. According to Swartz et al. (2014) genus of bacteria most commonly found in cattle and sheep are *Aggregatibacter* and *Streptobacillus*. In a study using Nellore cattle, Laguardia-Nascimento et al. (2015) found *Aeribacillus*, *Bacteroides*, *Bacillus*, and *Prevotella* to be among the most prevalent genera in the bacterial community in the vagina. The uterus is important in the maintenance of pregnancy. Verstraelen et al. (2015) examined the human uterine microbiome of reproductive age women and found that the uterus had a significant amount of bacterial species diversity and the most prevalent genus was *Bacteroides*.

The objective of this research was to assess the microbiome of the vagina, uterus, and embryo, and to determine the impacts of pregnancy status and treatment with a maternal pregnancy recognition antagonist. We hypothesized that location, pregnancy status and treatment with a maternal pregnancy recognition antagonist would result in significant differences in the bacterial microbiome of the reproductive tract in sheep.

MATERIALS AND METHODS

Animals and Treatments

New Mexico State University Institutional Animal Care and Use Committee approved all procedures associated with this experiment. Sixteen Rambouillet-cross ewes were used in this experiment. Controlled internal drug release inserts were used for 5 d and two injections of dinoprost tromethamine (5 mg intramuscular; Lutalyse: Pfizer, New York, NY) administered 4 h apart to synchronize ewe estrus. Ewes were mated with the ram until marked. Marked ewes were considered pregnant and randomly placed into treatment groups, either control (PBS, n=9) or treatment (AMD3100, n=7). Pregnant ewes

were administered AMD3100 which is an antagonist for chemokine receptor four signaling during early gestation and results in a disruption of fetal attachment. The treatments were administered in the uterus by surgically placed mini-osmotic pumps equipped with a catheter that was introduced into the lumen of the uterus (2 mL reservoir volume and pumping rate of 10 µl/h; Alzet 2ML1).

Sample Collection: Samples for microbiome analysis were obtained from three regions: vagina, uterus, and embryo. Upon collection 10 ewes were found to be not pregnant. Samples were collected and pregnancy status was noted. Using aseptic techniques, each location was swabbed with two sterile cotton swabs and the cotton swabs were placed into a 2-mL screw cap tube with 1.5 mL of less than 0.1 mM EDTA TE buffer. All ewes were anesthetized and vaginal samples were obtained prior to the removal of the reproductive tract. The reproductive tract was then removed using a mid-ventral laparotomy. Uterus was swabbed upon removal of the embryo. Embryos were placed on clean cutting board, then swabbed. Samples were stored at -80°C for subsequent DNA extraction. Ewes were euthanized by exsanguination.

Lab Analysis: The DNA was extracted from samples using a repeated beating plus column (RBB+C) method (Yu and Morrison, 2004). Briefly, samples were allowed to thaw at room temperature and 250 µl of TE with 1 cotton swab was placed in a sterile 2-mL screw cap tube with 0.4 g of zirconia beads (0.3 g of 0.1 mm beads and 0.1 g of 0.5 mm beads) and 1 mL of lysis buffer using the QIAmp DNA Stool Mini Kit (Qiagen, Valencia, CA). Subsequent steps in the DNA extraction protocol followed directions provided in the QIAmp DNA Stool Mini Kit (Qiagen, Valencia, CA). The quality and quantity of DNA was determined by a NanoDrop Spectrophotometer (Thermo Scientific, Marietta, OH).

Amplification and sequencing of the V4 variable region 16S rRNA gene was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA). Briefly samples were barcoded and PCR primers 515F/806R were used in a 28 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, with a final elongation step at 72°C for 5 minutes. A DNA library was prepared according to Illumina TruSeq DNA library preparation protocol. Sequencing was performed on a MiSeq (Illumina) following the manufacturer's guidelines. The PCR products were checked in a 2% agarose gel to determine application success and relative intensity of bands. Following PCR, all samples were pooled together in equal proportions based on molecular weight and DNA concentration. Samples were purified using calibrated Ampure XP beads. The DNA library was prepared by following Illumina TruSeq DNA library protocol, using purified PCR product. Sequencing was performed on a MiSeq following manufacturer's guidelines, and sequence data were processed using MR DNA analysis pipeline. Operational taxonomic units (OTUs) were generated from sequencing after removing < 150bp sequences. Final OTUs were clustered by 97% similarity and classified using BLASTn against a curated database derived from GreenGenes, RDPII and NCBI.

Statistical Analysis: Data was analyzed using Mixed procedure of SAS 9.3. Treatments included pregnant with PBS (PBSP), open PBS (PBSO), pregnant AMD3100 (AMDP), and open AMD3100 (AMDO). Model included effect of treatment, location, and treatment X location interaction. Single-degree of freedom contrasts were used to determine the effect of pregnancy on the composition of the microbiome. Statistical significance was determined at $P \leq 0.05$. Treatment means were calculated using the LSMEANS statement in SAS and when the model was significant treatment means were separated using PDIFF.

RESULTS AND DISCUSSION

Treatment had no effect on the microbiome composition ($P > 0.98$). However, the bacterial populations did differ according to sampling location ($P > 0.01$). In the vagina 47.2 $\pm$ 4.45% of the bacteria present were in the phylum *Firmicutes* ($P < 0.01$), 26.1 $\pm$ 5.62 % *Proteobacteria*, and 20.3 $\pm$ 2.71% *Actinobacteria*. While *Proteobacteria* was the dominant phyla in the uterus ($P < 0.01$) followed by *Firmicutes* and *Actinobacteria* making up 55.7 $\pm$ 5.62%, 28.7 $\pm$ 4.45% and 8.2 $\pm$ 2.71%, respectively. In support of the phylum data, the genus of bacteria present also differed between the uterus and vagina. C*orynebacterium* was the dominant genus in the vagina ($P < 0.01$) making up 11.1 $\pm$ 2.17% followed by 8.1 $\pm$ 0.53% *Finegoldia*. Limited and conflicting data is available on the role *Corynebacterium* in the vagina. Sobel and Chaim (1996), found 25% of women in their study to have *Corynebacterium* present in vaginal samples however it was not the predominant genus. Trost et al. (2010) associated *Corynebacterium* with pregnancy complications in women including late term abortion. Garza et al. (2015) found 1.37% *Corynebacterium* present in pregnant ewes but these authors did not note complications associated with the presence of *Corynebacterium*. In the uterus *Bradyrhizobium* was the prevailing genus ($P < 0.01$) making up 9.4 $\pm$ 1.11% of the bacterial population. In previous studies *Bradyrhizobium* has not been associated with uterine microbiome (Verstraelen et al., 2015) and it usually characterized as genus of Gram-negative soil bacteria, many of which fix nitrogen. *Bradyrhizobium* should be further investigated to determine role in uterine bacterial community or possible environmental contaminant.

Vagina phyla differed from Swartz et al. (2014) where the phyla *Bacteriodetes*, *Fusobacteria*, and *Proteobacteria* were most prominent in both cows and ewes. While *Proteobacteria* was prominent in the current study; *Bacterioidetes* and *Fusobacteria* were not significant. Our data agrees with Laguardia-Nascimento et al. (2015) who reported 40-50% *Firmicutes* and 5-25% *Proteobacteria*. Abundant genera differed from Laguardia-Nascimento et al. (2015) study found *Aeribacillus*, *Bacteroides*, and *Clostridium* to be the most abundant. Differences in results from Laguardia-Nascimento et al. (2015) and Swartz et al. (2014) could be caused by

differences in collection techniques. Laguardia-Nascimento et al. (2015) used a vulva wash of distilled water and 70% ethanol before sampling, vaginal samples were collected by introducing sterile saline to the vagina and aspirating the wash. Swartz et al. (2014) used a similar vaginal lavage technique however, vulvas were not washed before sample collection. *Bacteroidetes* was the predominant phylum found in the human uterus (Verstraelen et al., 2015), while *Bacteroidetes* was not significant in the present study. Verstraelen et al. (2015) harvested endometrial tissue and mucus by using a transcervical device (Tao Brush™ IUMC Endometrial Sampler; Cook OB-GYN, Bloomington, IN). Laguardia-Nascimento et al. (2015) and Swartz et al. (2014) and the current study found limited similarity to human vaginal microbiome when compared to ewe or cow vaginal microbiome indicating that more work is warranted in these species to ascertain the role of the vaginal microbiome in livestock fertility .

The role of pregnancy on reproductive tract and fetal microbiomes was also investigated. Nonpregnant ewes (PBSO and AMDO) versus pregnant (PBSP and AMDP) did not differ by phylum ($P > 0.14$) however, the genus *Finegoldia* was 7.5% greater in nonpregnant ewes ($P < 0.01$). *Finegoldia* is a Gram-positive, non spore forming obligate anaerobe commensal bacterium that has been found colonizing human skin and mucus membranes (Goto et al., 2008; Yuli and Finegold, 2011). However, it is also recognized as a pathogen responsible for various infectious diseases (Goto et al., 2008). *Finegoldia* has been associated with bacterial infections after surgical procedures (Moreuil et al., 2014). Garza et al. (2015) characterized the vaginal microbiome of pregnant and nonpregnant ewes, and did not report the presence of *Finegoldia* in nonpregnant ewes. The surgical procedure for this study could have introduced *Finegoldia* as a contaminant.

Effects of treatment were not observed on embryo microbiome on phylum ($P < 0.90$) or genus ($P < 0.88$) levels. There is very limited data on embryo microbiome. Further research is needed to investigate the bacterial microbiome of the vagina, uterus, and embryo in order to better understand the effects of the bacterial population on reproduction.

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Comparisons of two short duration estrous synchronization protocols on pregnancy rates to fixed-time AI[1]

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ABSTRACT: The objective of the current experiment was to compare pregnancy rate and estrus response between a 5-day or 6-day CO-Synch + CIDR synchronization protocol. Multiparous cows (n = 238) were assigned to either a 5-day CO-Synch + CIDR (5-Day) or a PG 6-day CIDR (6-Day) groups based on body weight and body condition score (BCS). Cows assigned to the 5-day protocol were given GnRH (100 µg i.m., Factrel) at the time of insertion of a controlled internal drug releasing device (CIDR; Eazi-Breed CIDR). Five d later CIDR was removed and PGF2α (25 mg i.m., Lutalyse) was given with an additional injection of PGF2α eight hours after CIDR removal. Cows assigned to the 6-day protocol were given an injection of PGF2α and three d later a CIDR was inserted and an injection of GnRH was given. Six d later CIDR were removed and PGF2α was given. Estrus detection aids were applied at CIDR removal. Cows were inseminated by fixed-time AI (FTAI) with conventional semen 72 h after CIDR removal, and GnRH was administered at the time of AI. At insemination, estrus status was categorized as positive (YES), unknown (NR) or negative (NO). Cows were divided into three groups and bulls were introduced 14 d post-insemination at a 1:50 ratio. Bulls were removed 60 d after FTAI and pregnancy was determined by transrectal ultrasound. Pregnancy diagnosis was confirmed by palpation 60 d after the bulls were removed. The AI and final pregnancy rates averaged 62.6% and 95.0%, respectively, and were similar (P < 0.7) between 5-Day and 6-Day protocol. There was no difference (P = 0.11) in the percentage of cows expressing estrus between the treatments (42.4% and 54.9% for 5-Day and 6-Day, respectively). Expression of estrus before FTAI increased (P < 0.05) AI pregnancy rates by 21%; however, it did not increase (P = 0.32) final pregnancy rates. There was no interaction (P = 0.11) among synchronization protocols and expression of estrus on AI pregnancy rates. In conclusion, expression of estrus increased pregnancy rates; however, there was no difference in pregnancy rates between synchronization protocols.

Key words: beef cows, estrous synchronization, fixed-time AI.

INTRODUCTION

Less than 10% of the beef herds in the US use AI (USDA APHIS, 2009). In commercial beef herds, AI is predominately used in heifers. However, estrous synchronization and fixed-timed AI (FTAI) of beef cows results in more cows calving earlier in the calving season and may increase uniformity in calves. Cow bred by fixed-timed AI followed with clean-up natural service weaned more kilograms of calf than cows bred using a traditional natural service system (Rodgers et al., 2012). Increasing the adoption of FTAI in commercial cow herds could improve productivity.

Several short-term synchronization protocols for FTAI in beef cows are currently recommended (Johnson et al., 2015). These include the 7-day CO-Synch + CIDR and 5-day CO-Synch + CIDR protocols (Figure 1); however, Perry et al. (2011) recently proposed a PG 6-day CIDR protocol. This protocol was designed as a combination estrus detection/fixed-time AI protocol, but it can be used for a pure FTAI protocol (Perry et al., 2012; Bridges et al., 2014).

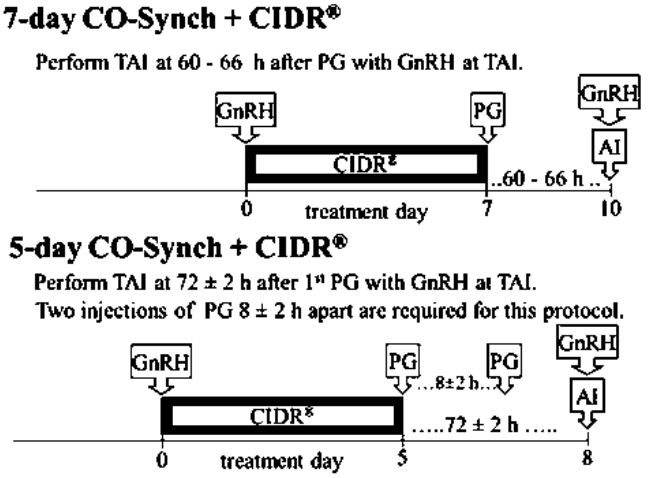

Figure 1. Currently recommended fixed-time AI protocols for use in beef cows. GnRH = 100 µg gonadorelin hydrochloride or gonadorelin diacetate tetrahydrate; PG = 25 mg $PGF_{2\alpha}$ or analog equivalent; CIDR = controlled internal drug release device (Eazi-Breed CIDR).

Expression of estrus is important after estrous synchronization as it can increase conception rates when compared to cows that did not express estrous (Richardson et al., 2016). Expression of estrus before FTAI in beef cattle resulted in increased follicular diameter and estrogen concentrations leading to increased conception rates (Perry

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et al., 2007). The 5-d CO-Synch + CIDR and PG 6-d CIDR protocols are designed to increase the length of follicular development during low progesterone exposure that may lead to increased estrogen concentrations.

Similar conception rates to FTAI have been found between 5-day CO-Synch + CIDR and PG 6-day CIDR in heifers (Bridges et al., 2014); whereas, a comparison of these protocols in cows demonstrated a slight advantage to the PG 6-day CIDR protocol (Perry et al., 2012).

The objectives of the current study were to: 1) determine if there was a difference in pregnancy rates and expression of estrus between a 5-day CO-Synch + CIDR and PG 6-day CIDR, and 2) add to the limited information about the efficacy of the PG 6-day CIDR system.

MATERIALS AND METHODS

Multiparous cows (n = 238) were assigned to either a 5-day CO-Synch + CIDR (5-Day) or a PG 6-day CIDR (6-Day) groups (Figure 2) based on body weight and body condition score. Cows assigned to the 5-Day protocol were given GnRH (100 µg i.m., Factrel, Zoetis, New York, NY) at the time of insertion of a controlled internal drug releasing device (CIDR; Eazi-Breed CIDR, Zoetis, New York, NY). Five d later CIDR was removed and PGF2α (25 mg i.m., Lutalyse, Zoetis, New York, NY) was given with an additional injection of PGF2α (25 mg i.m.) eight hours after CIDR removal. Cows assigned to the 6-Day protocol were given PGF2α and three d later a CIDR was inserted and an injection of GnRH was given. Six d later CIDR were removed and PGF2α was given. Estrus detection aids (Estrotect; Denver, CO) were applied at CIDR removal. In both treatments, cows were inseminated by professional technicians with conventional semen from one of six bulls 72 h after CIDR removal, and GnRH was administered at the time of AI. The time from CIDR removal to AI was recorded.

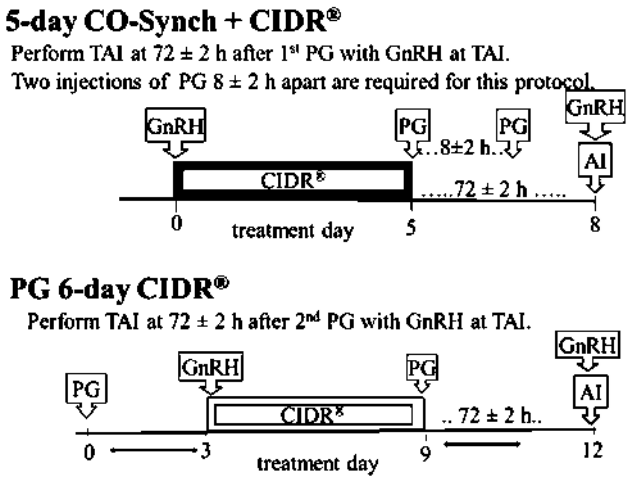

Figure 2. Diagrams of protocols used in the current study. GnRH = 100 µg gonadorelin hydrochloride or gonadorelin diacetate tetrahydrate; PG = 25 mg $PGF_{2\alpha}$ or analog equivalent; CIDR = controlled internal drug release device (Eazi-Breed CIDR).

At insemination, estrus status was categorized as positive (YES; activated patch), unknown (NR; lost or unrecorded patch) or negative (NO; inactivated patch). Cows were randomly divided into three groups and bulls were introduced 14 d post-insemination at a 1:50 ratio. Bulls were removed 60 d after FTAI and pregnancy was determined by transrectal ultrasound. Final pregnancy diagnosis was confirmed by palpation 60 d after the bulls were removed.

Results were analyzed by using a categorical model relating Yes/No response of final or AI pregnancy rate to the effects of treatment (5-Day, 6-Day) and patch (YES, NR, NO) using the procedure CATMOD.

RESULTS

There was no difference in AI (average 62.6%; P = 0.73) and final pregnancy (average 95.0%; P = 0.75) rates between 5-Day and 6-Day protocols (Figure 3). There was no difference (P = 0.11) in the expression of estrus between the treatments (Table 1). Expression of estrus before FTAI significantly increased (P < 0.05) AI pregnancy rates, however, it did not increase (P = 0.32) overall pregnancy rates (Table 2). There was no interaction (P = 0.11) among synchronization protocols and expression of estrus on AI pregnancy rates (Table 3).

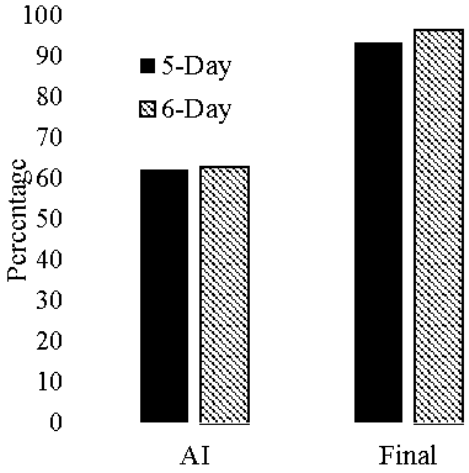

Figure 3. The effect of synchronization protocol on AI or final pregnancy rate. Cows assigned to the 6-Day protocol received an injection of PGF2α (25 mg; Lutalyse, i.m.) on d 0, an injection of GnRH (100 µg, Factrel, i.m) and insertion of a CIDR on d 3, and PGF2α (25 mg) injection and CIDR removal on d 9. Cows assigned to the 5-Day protocol received an injection of GnRH and insertion of a CIDR on d 0, and on d 5, an injection of PGF2α and CIDR removal, followed by a second injection of PGF2α 8 h later. All cows were time-inseminated 72 ± 2 h after CIDR removal. No effect of protocol (P > 0.01).

Table 1. The effect of synchronization protocol percentage (proportion) of cows expressing estrus[1] at time of AI.[2]

	Estrus Status		
Treatment[3]	Yes	NR	No
5-Day	42.4% (53/125)	20.0% (25/125)	37.6% (47/125)
6-Day	54.9% (62/113)	19.5% (22/113)	25.7% (29/113)

[1]Estrus status: Yes = detection aid activated, NR = detection aid missing, No = detection aid not activated.
[2]Effect of synchronization protocol (P = 0.11)
[3]Cows assigned to the 6-Day protocol received an injection of PGF2α (25 mg; Lutalyse, i.m.) on d 0, an injection of GnRH (100 µg, Factrel, i.m) and insertion of a CIDR on d 3, and PGF2α (25 mg) injection and CIDR removal on d 9. Cows assigned to the 5-Day protocol received an injection of GnRH and insertion of a CIDR on d 0, and on d 5, an injection of PGF2α and CIDR removal, followed by a second injection of PGF2α 8 h later. All cows were time-inseminated 72 ± 2 h after CIDR removal.

Table 2. The effect of estrus status[1] on pregnancy rate (proportion) to AI and AI + nature service.

	Estrus Status		
Pregnancy status	Yes	NR	No
AI	69.6%[a] (80/115)	68.1%[a] (32/47)	48.7%[b] (37/76)
Final	98.3 (113/115)	93.6 (44/47)	90.8 (69/76)

[1] Estrus status: Yes = detection aid activated, NR = detection aid missing, No = detection aid not activated.
[a,b] Within rows, different superscripts indicated effect of estrus status (P < 0.05).

Table 3. The effect of synchronization protocol and estrus status[1] on AI pregnancy rate (proportion).

	Estrus Status		
Treatment	Yes	NR	No
5-Day	77.4% (41/53)	60.0% (12/20)	46.8% (22/47)
6-Day	62.9% (39/62)	77.3% (17/22)	51.7% (15/29)

[1] Estrus status: Yes = detection aid activated, NR = detection aid missing, No = detection aid not activated.
[2]Interaction among protocol and estrus status (P = 0.11)
[3]Cows assigned to the 6-Day protocol received an injection of PGF2α (25 mg; Lutalyse, i.m.) on d 0, an injection of GnRH (100 µg, Factrel, i.m) and insertion of a CIDR on d 3, and PGF2α (25 mg) injection and CIDR removal on d 9. Cows assigned to the 5-Day protocol received an injection of GnRH and insertion of a CIDR on d 0, and on d 5, an injection of PGF2α and CIDR removal, followed by a second injection of PGF2α 8 h later. All cows were time-inseminated 72 ± 2 h after CIDR removal.

DISCUSSION

In the present study, FTAI pregnancy rates were similar between the two estrous synchronization protocols. Pregnancy rates to FTAI averaged 62% regardless of protocol. This pregnancy rate is similar to reports of the efficacy of the 5-day CO-Synch+CIDR protocol in postpartum beef cows (Kasimanickam et al., 2009; Whittier et al., 2013). However, reports on the efficacy of the PG 6-day CIDR protocol as a FTAI protocol for use in postpartum cows are more limited. Perry et al. (2012) reported a 9% increase in pregnancy rates to FTAI when using PG 6-day CIDR compared to 5-day CO-Synch + CIDR in postpartum cows; however, a meta-analysis found no differences in efficacy among 5 of the most common FTAI protocols (Richardson et al., 2016).

In agreement with multiple studies (Whittier et al., 2013; Richardson et al, 2016), cows that exhibited estrus before FTAI in the present study were 21% more likely to become pregnant to AI than cows that were not in estrus. Cows in estrus before FTAI may have larger follicles with ova that matured under a more favorable steroid environment, particularly elevated estrogen concentrations (Perry et al., 2007). Cows not expressing estrus before FTAI are induced to ovulate follicles when administered GnRH concomitant with AI. These prematurely ovulated follicles may result in decreased pregnancy rates; however, the resulting pregnancies in 35% to 50% of non-estrual cows may indicate follicular development had progressed sufficiently to warrant insemination of these cows.

Pregnancy rates to AI were also greater in cows without an estrus detection patch compared to cows not expressing estrus at FTAI. In most cases, these NR cows may have lost their estrus detection patches as a result of multiple mounts during estrus; however, failure of patch retention for other reasons could not be ruled out. In the present study, the excellent AI pregnancy rates indicate that many of the cows without patches at FTAI had been in estrus.

Although 12% more cows in the 6-Day protocol were in estrus before FTAI compared to 5-Day cows, the number of cows used in this study provided insufficient power to detect a difference in estrus expression between treatments. Previous studies provide limited information on estrus response before FTAI when using the PG 6-day CIDR system. Richardson et al., (2016) reported a low (39%) estrus response rate before FTAI, whereas, another study did not report estrus response rates before FTAI (Perry et al., 2012). In the present study, both protocols resulted in a majority of cows expressing estrus before FTAI.

The PG 6-day CIDR protocol may enhance synchrony of follicular wave emergence as a result of increased ovulation rate after the initial GnRH injection compared to 5-day CO-Synch + CIDR (Perry et al., 2012). This increase response to initial GnRH may result in greater pregnancy rates (Perry et al., 2012). However, in the present study, the weak tendency towards greater numbers of cows in estrus before FTAI in the 6-Day group did not translate into increased pregnancy rates.

One of the barriers to adoption of FTAI is the number of times cattle must be handled. Both of the protocols are effective in synchronizing postpartum cows for FTAI and

result in acceptable pregnancy rates. However, both of the protocols require cows to go through the chute four times. However, the 5-Day protocol only requires cows and calves be gathered and sorted three times compared to four gather/sort procedures for 6-Day. This is because cows and calves remain separate in the 5-Day protocol between the first and second prostaglandin injection. One of the goals with FTAI programs is to only have cattle go through the chute three times including AI. Producers must realize a pregnancy rate advantage to compensate for four trips through the chute.

For commercial operations, the primary benefit to AI is an increase in kilograms of calf weaned per cow. This increase in production appears to be a function of more calves born earlier in the calving season, improved genetic merit and increased calf survival (Patterson et al., 2006, Rodgers et al., 2012). Additional economic comparisons need to be conducted in conjunction with FTAI studies.

IMPLICATIONS

Fixed-time AI protocols offer an opportunity for commercial producers to take advantage of the genetic and economic improvement of AI while reducing labor associated with AI. The 5-day CO-Synch + CIDR and PG 6-day CIDR fixed-time AI protocols are equally efficacious in estrus response and pregnancy rates. Pregnancy rates to AI in the present study of over 60% are compatible goals of a commercial AI program.

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Effect of delayed insemination of non-estrual beef heifers following a 7-d-CO-Synch plus controlled internal drug release (CIDR) insert timed artificial insemination protocol

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ABSTRACT: Synchronizing estrus before AI is an effective way to shorten the calving season, and increasing the number of pregnancies per AI may lead to greater use and acceptance of synchronization protocols among beef producers. Our objective was to determine if pregnancy rates to fixed-time AI (**FTAI**) would be improved by delaying insemination in heifers not expressing estrus before FTAI in a 7-d CO-Synch + controlled internal drug release (**CIDR**) estrous synchronization protocol. Four hundred sixty-five yearling beef heifers across three locations of commercial and purebred herds were given 100 µg of GnRH (Cystorelin) i.m. and a CIDR insert (Zoetis; 1.38 g of progesterone) on d 0. On d 7 CIDR inserts were removed and all heifers received 25 mg of $PGF_{2\alpha}$ i.m. (Lutalyse; Zoetis) and were fitted with an estrous detection patch (Estrotect; Rockway, Inc.). Heifers were placed in one of three treatment groups based on estrous detection patch color at 48 h after $PGF_{2\alpha}$: 1. Estrus-Red 48 h (n = 180) - heifers displayed estrus as indicated by red estrous detection patch and were given GnRH (100 µg i.m. Cystorelin) and inseminated at 48 h after $PGF_{2\alpha}$, 2. Non-Estrus-Gray 48 h (n = 137) - heifers did not display estrus by 48 h after $PGF_{2\alpha}$ and were given GnRH (100 µg i.m. Cystorelin) and inseminated at 48 h after $PGF_{2\alpha}$, and 3. Non- Estrus Delayed- Gray 56 h (n = 148) - heifers did not display estrus by 48 h after $PGF_{2\alpha}$ and were given GnRH (100 µg i.m. Cystorelin) at 48 h and inseminated at 56 h after $PGF_{2\alpha}$. Pregnancy data were analyzed using SAS PROC GLIMMIX with treatment as a fixed effect, herd as a random variable, and heifer as the experimental unit. By 48 h after $PGF_{2\alpha}$, 38.7% of all heifers were in estrus (as indicated by a red estrous detection patch). Pregnancy rate to AI was greatest for Estrus-Red 48 h heifers (67.8%; $P < 0.0001$) as compared to heifers in the Non- Estrus- Gray 48 h (39.4%) and Non-Estrus Delayed- Gray 56 h (42.6%) groups. Among heifers not expressing estrus before FTAI, delayed insemination achieved a similar ($P = 0.83$) percent of pregnancies (Non-Estrus Delayed-Gray 56 h; 42.6%) as compared to Non-Estrus-Gray 48 h heifers (39.4%). Delaying insemination by 8 h in heifers not displaying estrus by 48 h after $PGF_{2\alpha}$ did not improve pregnancy rates to AI.

Key words: beef heifers, estrous synchronization, fixed-time artificial insemination

INTRODUCTION

Reproductive performance in cattle is considered to be the most economically important trait and is essential for the success of an operation (Wiltbank, 1990). In the last decade, fixed-time AI (FTAI) in the beef cattle industry has increased in popularity (National Animal Health Monitoring System, 2009), in part, because of the ability to achieve similar pregnancy rates to AI as compared with AI following detection of estrus (Patterson et al., 2003). Unfortunately, even with this increase, a recent survey (National Animal Health Monitoring System, 2009) reported only 7.6% of beef cattle producers use AI and only 16.3% of beef heifers were artificially inseminated. The most common reasons for not using this reproductive technology is it requires time and labor. In order to increase the use of estrous synchronization and AI, protocols need to continue to decrease the number of animal handlings and increase the percent of pregnancies to AI within a herd.

Fixed-time AI has allowed for the reduction of time and labor required for estrous detection. Unfortunately, producers still encounter heifers not expressing estrus at time of AI. Several studies have shown that pregnancy rate to FTAI varies with estrous expression with females expressing estrus before FTAI having greater pregnancy rates (Perry et al., 2005; Perry et al., 2007; Richardson et al., 2016). When females are inseminated at the fixed times between 54 and 66 h as recommended by protocols from Applied Reproductive Strategies in Beef Cattle (2015), estrous expression varies. A previous study by Busch et al. (2008) found that approximately 50% of cows did not express estrus before FTAI at 54 h in a 7-d-CO-Synch + controlled internal drug release (CIDR) protocol, and a similar result was observed in beef heifers in a 14-d CIDR PG protocol (Mallory et al., 2011).

A previous study compared the effects of delaying insemination until 20 h after GnRH in beef females not expressing estrus in a 14-d CIDR PG protocol (Thomas et al., 2014). It was found that pregnancy rates improved ($P = 0.02$) by delaying insemination in non-estrus heifers by 20 h after GnRH compared to insemination at the standard time (49 versus 34%). This study did not compare the effects of pregnancy rates in heifers in a 7-d-CO-Synch + CIDR protocol. Therefore, the objective of this study was to determine if pregnancy rates could be improved in beef heifers not expressing estrus before FTAI by delaying insemination in a 7-d-CO-Synch + CIDR protocol.

MATERIALS AND METHODS

The experimental procedures were approved by Kansas State University Animal Care and Use Committee. Estrus was synchronized using the 7-d-CO-Synch + CIDR FTAI protocol (Fig. 1) across 3 locations of commercial and purebred beef heifers (n = 465). Heifers were given GnRH (100 µg, i.m.; Cystorelin; Merial, Athens, GA) and an Eazi-Breed CIDR insert (1.38 g of progesterone; Zoetis Florham

Park, NJ) on d 0. The CIDR inserts were removed 7 d later and the heifers received an injection of $PGF_{2\alpha}$ (25 mg, i.m.; Lutalyse; Zoetis) and estrous detection patches (Estrotect; Rockway, Inc., Spring Valley, WI)were applied. All heifers were administered GnRH on d 9 at 48 h after $PGF_{2\alpha}$ regardless of estrous expression. Estrus was defined as >50% of the gray rub-off coating on the Estrotect patch being removed exposing red color underneath the rub-off coating. Heifers were placed in one of three treatment groups base on estrous detection patch color at 48 h after $PGF_{2\alpha}$: 1. Estrus-Red 48 h (n = 180) – heifers displayed estrus as indicated by red estrous detection patch and were given GnRH (100 µg i.m. Cystorelin) and inseminated at 48 h after $PGF_{2\alpha}$, 2. Non-Estrus-Gray 48 h (n = 137) - heifers did not display estrus by 48 h after $PGF_{2\alpha}$ and were given GnRH (100 µg i.m. Cystorelin) and inseminated at 48 h after $PGF_{2\alpha}$, and 3. Non-Estrus Delayed- Gray 56 h (n = 148) - heifers did not display estrus by 48 h after $PGF_{2\alpha}$ and were given GnRH (100 µg i.m. Cystorelin) at 48 h and inseminated at 56 h after $PGF_{2\alpha}$. Estrotect patches remained on all heifers that did not display estrus at 48 h after $PGF_{2\alpha}$, and estrous expression of these heifers was recorded at 56 h after $PGF_{2\alpha}$.

Pregnancy Diagnosis

At 30 to 50 d post AI, transrectal ultrasonography (5MHz transrectal transducer, Aloka 500V, Wallingford, CT) was used to confirm pregnancy. Pregnancy to AI was determined by the presence of uterine fluid and an embryo.

Statistical Analysis

Data were analyzed as a completely random design using the GLIMMIX procedure of SAS (SAS Enterprise Guide 4.3; SAS Inst. Inc., Cary, NC). Heifer served as the experimental unit, treatment was the fixed effect, and herd was a random variable. P-values ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Pregnancy rates to FTAI based on estrous response and treatment are shown in Table 1. The interval from $PGF_{2\alpha}$ to FTAI for the delayed AI was approximately 8 h. There was an effect of treatment on pregnancy rate to AI with heifers expressing estrus before FTAI (Estrus-Red 48 h 67.8%) having a greater ($P < 0.001$) pregnancy rate than heifers that were not expressing estrus at FTAI (Non-Estrus- Gray 48 h; 39.4%). These results are similar to those reported by Richardson et al. (2016). Richardson et al. (2016) compared multiple AI synchronization programs through a meta-analysis and determined that females detected in estrus before FTAI, regardless of the estrous synchronization protocol, had greater pregnancy success than heifers that did not express estrus before FTAI. Females exhibiting estrus before FTAI may have attained proper levels of estradiol to provide a more optimal uterine and reproductive tract environment for fertilization and pregnancy (Perry and Perry, 2008).

We hypothesized that by delaying insemination in heifers not expressing estrus at FTAI, we are allowing for a greater proportion of heifers to come into estrus and develop an optimal uterine environment for AI because of higher concentrations of circulating estradiol (Allrich, 1994). A recent study conducted by Thomas et al. (2014) found that delaying insemination by 20 h for heifers not expressing estrus in a 14-d CIDR PG protocol improved pregnancy rates compared to heifers not expressing estrus that were inseminated at the standard time (49 vs. 34%). During the 20 h delay, 54% of heifers expressed estrus, and the pregnancy rate increased ($P < 0.0001$) for these heifers compared to the heifers that did not express estrus before FTAI (66 vs. 29%). In the present study, we compared the effects of delaying insemination in heifers in a 7-d-CO-Synch + CIDR protocol and no increase ($P = 0.83$) was observed in pregnancy rate of heifers not expressing estrus (Non- Estrus- Gray 48 h [39.4%] and Non-Estrus Delayed-Gray 56 h [42.6%]). It was speculated that the results from this study suggest a favorable female reproductive tract environment is the most important factor to consider to improve pregnancy rates.

Another study that supported our hypothesis compared the effects of TAI at 56 and 72 h in a 5-d-CO-Synch + CIDR protocol in beef heifers (Kasimanickama et al., 2012). It was found in this study that pregnancy rate was greater ($P < 0.0001$) for heifers inseminated at 56 h after CIDR removal compared to heifers inseminated at 72 h after CIDR removal (66.2 vs. 55.9%). It was noted, however, that a greater ($P < 0.05$) proportion of heifers expressed estrus before insemination at 72 h compared to 56 h (82.4 vs. 73.1%). In the present study, the cumulative percentage of heifers expressing estrus at 48 h after $PGF_{2\alpha}$ was 38.7%. Of the 61.3% (285/465) of heifers that did not express estrus by 48 h after $PGF_{2\alpha}$, an additional 102 heifers (35.8%) expressed estrus by 56 h after $PGF_{2\alpha}$. Of the heifers in the Non-Estrus- Gray- delayed 56 h treatment, 37.8% (56/148) showed estrus at 56 h, and 62.1% (92/148) of the heifers did not express estrus. Of the heifers that expressed estrus, there was a 51.8% (29/56) pregnancy rate, and 36.9% (34/92) pregnancy rate for the heifers that did not express estrus.

Ovulation in females expressing estrus is caused by a surge of LH at the time of estrus. This surge of LH occurs because of a surge of GnRH. By giving exogenous GnRH 8 h before delayed insemination in heifers not expressing estrus (Non-Estrus- Gray- delayed 56 h), we are causing those females to release an endogenous surge of LH and ovulate without first expressing estrus. Because estradiol is not being secreted at greater levels, this decreases the chance of having a favorable reproductive tract environment, and therefore potentially decreases the chance of pregnancy success.

The increase in the number of heifers expressing estrus 8 h after GnRH causes us to question the benefit and effectiveness of administering GnRH to the heifers that have not expressed estrus before time of AI. We assume that the administration of GnRH to heifers not expressing estrus would actually limit the expression of estrus. The LH surge caused by the administration of GnRH should cause ovulation, and therefore eliminate the chance for an

increase in estradiol from positive feedback. We speculate that perhaps the magnitude of the LH surge may not be great enough to cause luteolysis and ovulation.

Synchronization of the estrous cycle has the potential to shorten the calving season, and increase calf uniformity. The development of FTAI has eliminated time and labor required for estrous detection. Expression of estrus before FTAI has been shown to increase pregnancy rates in females, and use of estrous detection patches to determine estrous activity has also helped to eliminate these requirements. We did not see an improvement in pregnancy rates in this study; however, we speculate that an even longer delay in GnRH administration and AI for heifers not expressing estrus will result in increased pregnancy rates.

IMPLICATIONS

Delaying insemination to 56 h after $PGF_{2\alpha}$ in non-estrual beef heifers in a 7-d-CO-Synch + CIDR protocol did not improve pregnancy rates. Estrus-detection patches, however, may be a valuable management tool that enables producers to divide heifers into groups based on estrous expression, with minimal time and labor input. It is possible that it may be more cost and time effect for producers to inseminate only the heifers that are displaying estrus at time of AI when utilizing FTAI protocols.

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Table 1. Pregnancy rate of heifers after fixed-time artificial insemination (FTAI) within treatment[1]

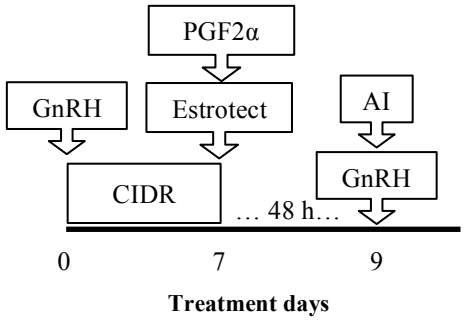
	Pregnancy Rate to FTAI[3]	
Estrous response[2]	Proportion	%
Estrus 48 h	122/180	67.8[a]
Non-Estrus 48 h	63/148	42.6[b]
Non- Estrus 56 h	54/137	39.4[b]
Total	239/465	51.4

[a,b]Pregnancy rates with different superscripts within columns are different, P<0.0001.

[1]Heifers received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 µg, intramuscular [i.m.]) on d 0. The CIDR insert was removed and $PGF_{2\alpha}$ (25 mg, i.m.) was administered on d 7. At 48 h after CIDR insert removal and $PGF_{2\alpha}$, heifers received GnRH (100 µg, i.m.) and were divided based off estrous expression. Heifers expressing estrus (Estrus-Red 48 h), and one-half of the heifers not expressing estrus (Non-estrus-Gray 48 h) were inseminated at time of GnRH. The other one-half of heifers not expressing estrus at 48 h (Non- Estrus Delayed- Gray 56 h) were inseminated 56 h after CIDR insert removal and $PGF_{2\alpha}$.

[2]Pregnancy rate to FTAI was determined by ultrasound 30 to 60 d after AI.

7-d-CO-Synch + CIDR



Delayed insemination for non-estrous heifers

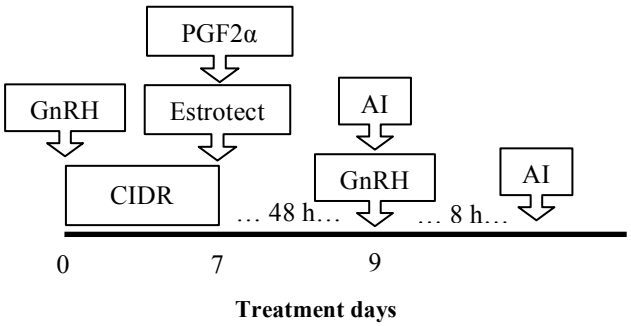


Figure 1. Treatment schedule for the 7-d-CO-Synch + controlled internal drug-release (CIDR) protocol. Heifers in each treatment received a controlled internal drug-release (CIDR) insert (1.38 g progesterone) and were administered GnRH (100 µg, intramuscular [i.m.]) on d 0. The CIDR insert was removed and $PGF_{2\alpha}$ (25 mg, i.m.) was administered on d 7. At 48 h after CIDR insert removal and $PGF_{2\alpha}$, heifers received GnRH (100 µg, i.m.) and were divided based off estrous expression. Heifers expressing estrus (Estrus-Red 48 h), and one-half of the heifers not expressing estrus (Non-estrus-Gray 48 h) were inseminated at time of GnRH. The other one-half of heifers not expressing estrus at 48 h (Non- Estrus Delayed- Gray 56 h) were inseminated 56 h after CIDR insert removal and $PGF_{2\alpha}$.

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Effect of prostaglandin administration after ram exposure on ewe reproductive efficiency

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ABSTRACT: A 2-yr experiment was conducted to determine the effect of a single injection of prostaglandin after ram turnout on ewe estrous synchronization. Rambouillet ewes (n=100; yr 1 = 52; yr 2 = 48) at New Mexico State University West Sheep Unit were stratified by age and BW and assigned to 1 of 3 treatments: untreated (**CON**; n = 33); 12-d CIDR insert (**CIDR**; n = 33); or 1 injection of prostaglandin at d 2.5 (**1PG**; n= 34) after rams were placed with ewes. Ewes were exposed to rams at CIDR insert removal (d 0) for a 35-d breeding season. Ewes were observed twice daily to determine estrus. A greater ($P \leq 0.01$) number of CIDR ewes were bred in the first 3 d of the breeding season (82%), compared with 1PG (35%) or control (21%) ewes. Moreover, there was an increased ($P \leq 0.01$) number of CIDR ewes bred in the first 4 d (94%) compared to 1PG (50%) ewes, both of which had an increased ($P \leq 0.01$) number of ewes bred compared to control ewes (21%). Both CIDR (94%) and 1PG (73.5) ewes had an increased number ($P \leq 0.01$) of ewes bred in the first 5 d compared to control (33%) ewes. As expected, CIDR-treated ewes had a shorter time (2.2 d) to breeding, than 1PG treat ewes (4.9 d) and control ewes took longer to breed than both CIDR and 1PG ewes (8.1 d) ($P \leq 0.01$). Lambing and weaning data have not yet been collected for yr 2. In yr 1 the number of lambs born per ewe and kg of lamb weaned per ewe was not different ($P \geq 0.33$) between treatments. Based on these data, utilizing a single injection of PG 2.5 d after ram turnout resulted in similar pregnancy rates at d 5 of the breeding season when compared with CIDR-treated ewes suggesting in a confinement setting the 1PG synchronization protocol could potentially be utilized as a less expensive method of synchronization. Additional information will be collected to determine the effects of synchronization protocol on post-lambing data to determine efficacy of the treatments. Moreover, more research is needed to determine the efficacy of the proposed synchronization protocol in a range production environment.

Key words: reproduction, sheep, synchronize

INTRODUCTION

In animal production systems, estrous synchronization is utilized to condense the breeding season, create a more uniform group of offspring, and induce estrus in anestrous females (Patterson et al., 2003; Dixon et al., 2006, Holm et al., 2008; Fierro et al., 2013). Progestin based synchronization protocols utilizing an intravaginal controlled internal drug release (CIDR) device are the most widely utilized synchronization protocols in ewes. The estrous cycle is manipulated by extending the luteal phase. Upon removal of the device, progesterone (P4) concentrations decline allowing for initiation of estrus (Wildeus, 2000). Prostaglandin based protocols function to shorten the luteal phase by lysing the corpus luteum (CL). Previous research has established that administration of prostaglandin (PG) between d 5 and 14 will induce luteolysis (Acritopoulo and Haresign, 1980). Advantages of PG based compared with progestin based protocols, include reduced labor inputs, ease of administration, and cost (Fierro et al., 2013). A major disadvantage of PG based protocols is the animal must have returned to estrous, allowing for lysis of the CL. Another disadvantage is that PG is not currently approved for use in sheep.

A single injection of PG 4.5 d after bull turnout has been proposed as a viable synchronization protocol in cattle (Whittier et al., 1991; Larson et al., 2009). The mechanism allowing for the success of this system results from the CL being unresponsive to PG for at least 96 h after ovulation (Larson et al., 2009). The ovine CL is refractory to treatment with PG up to 3 d postovulation (Rubianes et al., 2003). Therefore, exposing ewes to rams 2.5 d prior to PG administration would allow rams to breed ewes in estrus, while not impacting CL development.

The objective of this study was to compare estrus response and reproductive efficiency of the single injection prostaglandin synchronization protocol with the CIDR synchronization protocol.

MATERIALS AND METHODS

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

Animals and Treatments

Over a 2-yr period, 100 (yr 1 = 52; yr 2 = 48) Rambouillet ewes were blocked by age and BW and randomly assigned to 1 of 3 treatments: untreated (**CON**; n

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= 33); 12-d CIDR insert (EAZI-BREED CIDR, Zoetis Animal Health, Florham Park, NJ; **CIDR**; n = 33); and 1 injection of 2 mL prostaglandin (Lutylase, Zoetis Animal Health) at d 2.5 (**1PG**; n= 34) after ram turnout. Prior to treatment initiation, ewes were isolated from rams. Rams were placed with ewes on d 0 at a ewe to ram ratio ≤17:1. Marking paint (Raddle powder, Premier 1, Washington, IA) was applied to rams every 3 d as needed. Ewes were exposed to rams at CIDR insert removal (d 0) for a 35-d breeding season. Ewes were observed twice daily to determine estrus. Observation of breeding marks at 0730 h and 1700 h starting on d 0, was used to determine estrus.

Blood Collection

Beginning on d -3 and continuing through d 6 after CIDR removal blood samples were collected daily before morning feeding at 0730 h. Blood samples were collected from a random subset of ewes in yr 1 (n= 24; CON = 8; CIDR = 8; 1PG = 8) and on all ewes in yr 2 via jugular venipuncture into serum separator vacuum tubes (Corvac, Kendall Healthcare, St. Louis, MO). Samples were allowed to clot at room temperature for 30 min then subjected to centrifugation (1200 x g for 20 min at 4 ºC). Serum was harvested and stored at -20ºC until assayed. Serum progesterone concentrations were quantified by RIA using components of a solid phase kit (MP Biomedicals, LLC, Santa Ana, CA) and modified for use in ruminant serum as reported by Schneider and Hallford (1996). Intra- and inter-assay coefficients of variation were less than 15%.

Postpartum Measurements

Following the end of the breeding period, ewes were maintained as a single group during gestation. Ewes were maintained on a ration formulated to meet or exceed NRC (2007) requirements for each stage of production. In yr 1 pregnancy was confirmed via blood sample collected via jugular venipuncture. In yr 2 pregnancy was confirmed via abdominal ultrasonography. Ewes were sheared approximately 2 mo prior to lambing and vaccinated against *Clostridium perfringens* type C and D and *Clostridium tetani* (Bar Vac CD/T, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). At birth, lambs were individually identified using ear tags. Lamb birth weight, sex, and type of birth was recorded. On d 2, tails were docked and lambs received 1 mL intramuscular injections of BO-SE (Schering-Plough Animal health, Union, NJ), which contained 1 mg of selenium and 68 USP of vitamin E. Lambs and dams were kept in small pens for 1 to 2 wk before returning to the main flock. A corn based creep feed was introduced to lambs at approximately 10 d of age and remained until weaning. At approximately 30 d of age, lambs were vaccinated against *Clostridium perfringens* type C and D and *Clostridium tetani* (Bar Vac CD/T, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). Male lambs were also castrated via banding at this time. Lambs were weaned and weighed at approximately 60 d of age.

Statistical Analysis

Data were analyzed using the PROC MIXED and GLIMMIX procedures in SAS (SAS Inst., Inc., Cary, N.C.). For reproductive and lambing data, animal was considered the experimental unit with estrous synchronization treatment assigned as a fixed effect. Initial statistical analysis included year as a fixed effect and the treatment x year interaction. The treatment x year interaction was not significant and was removed from the model and year considered a random effect. Serum progesterone response to estrous synchronization treatment were subjected to repeated measures ANOVA for a split-plot design with ewe considered the experimental unit. Treatment was included in the main plot and treatment x day interactions included in the sub plot. Analysis of P4 concentrations were conducted using the MIXED procedure of SAS. A P-value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

Animal Performance

No trt x yr effect was detected for reproductive performance or lambing data therefore just the main effect of estrous synchronization will be discussed. Ewe reproductive performance is reported in Table 1. A greater ($P \leq 0.01$) proportion of CIDR ewes were bred in the first 3 d of the breeding season (82%), compared with 1PG (35%) ewes or control (21%) ewes. Additionally, there was an increased (P ≤ 0.01) proportion of CIDR ewes bred in the first 4 d of the breeding season (94%) compared with 1PG (50%) or control (21%) ewes. Both CIDR (94%) and 1PG (74%) ewes had a greater ($P \leq 0.01$) proportion of ewes breed in the first 5 d of the breeding season compared with control (33%) ewes. Similarly, Gonzalez-Bulnes et al. (2005) reported no difference in estrus response to ewes synchronized with either a progstaglandin or progestin based protocol (81.5% and 72.4% respectively). As expected, the number of days from ram turnout to breeding was shorter ($P \leq 0.01$) for CIDR (2.2 d), than for 1PG (4.9 d) ewes or control (8.1 d) ewes. Similarly, d to breeding was shorter ($P \leq 0.01$) for 1PG compared with CON ewes. Control ewes had an increased (P ≤ 0.01) Julian breeding date (293 d) compared with 1PG (290 d) ewes or CIDR (287 d) ewes. However, overall pregnancy rates did not differ among treatments ($P = 0.20$).

Serum P4 concentrations are reported in Fig. 1. There was a significant trt x day interaction for serum P4 concentrations. Serum P4 concentrations were similar among treatments from d -3 to 0 ($P \geq 0.20$). As expected, P4 concentrations for CIDR-treated ewes were less than both 1PG and CON on d1 ($P < 0.001$) of the breeding season. The

reduction in P4 concentrations can be attributed to the removal of the exogenous progestin on d 0, and is similar to other findings (Dixon et al., 2006; Benavidez et al., 2007) reporting decreased P4 concentrations after CIDR removal. Serum P4 concentrations remained elevated in 1PG ewes through d 2 and were similar ($P > 0.05$) to CON ewes. Treatment of PG occurred at d 2.5, resulting in reduced P4 concentrations on d 3. Blood serum P4 concentrations in 1PG ewes on d 3 tended to be similar ($P = 0.08$) to those of CIDR ewes at d 0, indicating CL lysis in ewes not previously bred. Effectiveness of PG treatment is verified by approximately 60% of 1PG treated ewes responding to synchronization within 72 h of PG administration. These data agree with Acritopoulou and Haresgin (1980) reporting 50% of ewes treated on d 3 of the estrous cycle responded to $PGF_{2\alpha}$, utilizing a similar dose of PG (10 mg) as the current study.

Postpartum Measurements

Lamb production data are summarized in Table 2. Lambing and weaning data have not been collected for yr 2. In yr 1 there was no difference ($P \geq 0.47$) in the Julian lambing date between the 3 treatments despite the CIDR ewes breeding 2.7 and 5.9 d earlier than 1PG and control ewes respectively. Additionally, there was no difference ($P \geq 0.12$) in gestation length among treatments. The number of lambs born per ewe and kilograms weaned per ewe were both similar ($P \geq 0.33$) between all treatments.

Ewes treated with 1 injection PG had similar estrous response rates at d 5 of the breeding season compared with CIDR-treated females, suggesting that incorporation of the single injection of PG 2.5 d after ram turnout may be a more economically feasible means of synchronizing ewes in a farm flock environment. More information is needed to determine the efficacy of the protocol on range sheep production and dose requirements to ensure complete CL regression within a timely manner.

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Table 1. Effect of synchronization system on reproduction in Rambouillet ewes

Item	CON[1]	CIDR[2]	1PG[3]	SEM	*P*-value
n	33	33	34	-	-
Age, yr	2.7	2.6	2.7	0.3	0.98
Julian breeding date, d	293	287	290	4.6	<0.01
Days to breeding, d	8.1	2.2	4.9	0.7	<0.01
Bred first 5 days, %	33[a]	94[b]	74[b]	7.1	<0.01
Bred first 4 days, %	21[a]	94[b]	50[c]	7.1	<0.01
Bred first 3 days, %	21[a]	82[b]	35[a]	7.5	<0.01
Final pregnancy rate, %	94	91	100	5.9	0.20

[a-c]Means with different superscripts differ $P \leq 0.05$.

[1] CON = untreated ewes.

[2] CIDR = ewes received a 12 d CIDR insert.

[3] 1PG = ewes received a single injection of prostaglandin 2.5 d following ram turnout.

Table 2. Effect of synchronization system on lamb production in Rambouillet ewes

Item	CON[1]	CIDR[2]	1PG[3]	SEM	*P*-value
n	17	17	18	-	-
Lambing date, J	86	84	84	1.7	0.47
Number of lambs per ewe	1.5	1.3	1.6	0.2	0.33
Adjusted kg weaned per ewe[4], kg	22.0	24.1	22.3	2.7	0.83
Gestation length, d	151.5	156.2	154.4	1.6	0.12

[1] CON = untreated ewes.

[2] CIDR = ewes received a 12 d CIDR insert.

[3] 1PG = ewes received a single injection of prostaglandin 2.5 d following ram turnout.

[4]Adjustment based on factors from (Scott, 1977).

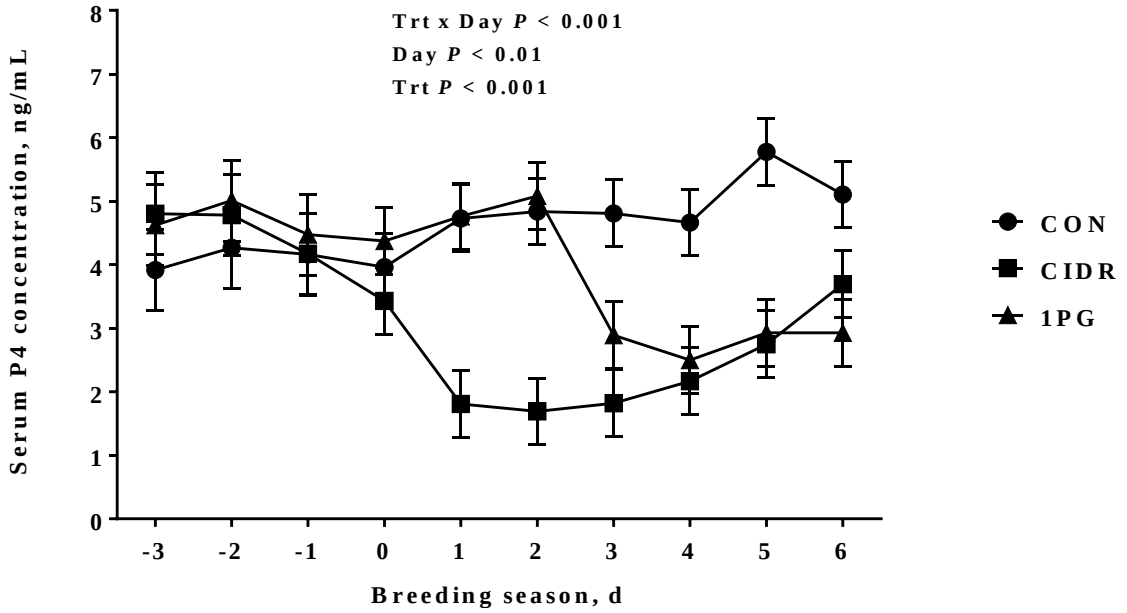

Figure 1. Effect of estrous synchronization protocol CON (no synchronization), CIDR (12-d CIDR, removed d 0) or 1 injection PG (d 2.5 of breeding season) on serum progesterone (P4) concentration in Rambouillet ewes just prior to and after ram turnout (d 0).

PRODUCTION, MANAGEMENT, AND THE ENVIRONMENT

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Effects of early or conventional weaning on beef cow and calf performance in pasture and drylot environments

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ABSTRACT : Spring-calving beef cows (initial BW = 599 ± 54.5 kg; initial BCS = 5.5 ± 0.54) and calves (initial BW = 204 ± 26.7 kg; 153 ± 15 d of age) were assigned randomly to 1 of 4 weaning treatments: weaning at 153 d of age followed by 56 d of limit feeding in confinement (**E-D**), confinement of cow and calf together for a 56-d period of limit feeding followed by weaning at 209 d of age (**C-D**), weaning at 153 d of age followed by a 56-d grazing period (**E-P**), and a 56-d grazing period with cow and calf together followed by weaning at 209 d of age (**C-P**). Calves assigned to E-D and C-D were fed a concentrate-based diet at 2.5% of BW, whereas cows assigned to E-D were fed a forage-based diet at 1.6% of BW. Cows assigned to C-D were offered the diet fed to E-D cows at 2.0% of BW. All cows and calves were limit fed common diets for 7 d at the end of our study to equalize gut fill. Calves ADG were influenced by diet and weaning treatments ($P \leq 0.03$). In general, calves managed in confinement and fed concentrate-based diets (i.e., E-D and C-D) had greater ADG than unsupplemented calves maintained on pasture (i.e., E-P and C-P). Cow BW and BCS change (d 0 to 63) were influenced by diet and weaning status ($P \leq 0.05$). Non-lactating cows maintained on pasture had lesser BW loss than other treatments, whereas non-lactating cows fed in confinement had lesser BCS on d 63 and greater BCS loss from d 0 to 63 than other treatments. Conversely, rump-fat depth on d 63 was greater ($P < 0.01$) for non-lactating cows maintained on pasture than for lactating cows in either pasture or drylot environments. Similarly, change in rump-fat depth was greatest ($P < 0.01$) for non-lactating cows on pasture and least for lactating cows in both pasture and drylot environments. Results were interpreted to indicate that weaning at 153 d of calf age spared cow BW and rump fat compared to weaning at 209 d of calf age. Performance of cows appeared to be similar when either limit-fed under drylot conditions or pastured without supplement. Conversely, calf performance was greater in confinement than on pasture.

Keywords: beef cows, concentrate, early weaning, pasture

Introduction

With widespread drought across the Midwest in recent years, pasture availability and productivity have been reduced. This, coupled with increasing land prices and lease rates, has prompted the evaluation of alternative management strategies that decrease grazing pressure on perennial pasture or reduce feed and pasture costs. Weaning early and moving cows from pasture to a drylot environment is used commonly for reducing grazing pressure on perennial pastures. A premature end to lactation reduces cow nutrient requirements and reduces grazing pressure. Removal of the calf further reduces grazing pressure, as calves are significant consumers of forage DM during mid and late lactation (Boggs et al., 1980). The combination can be used to extend grazing by 0.4 d for each d weaning is executed earlier than normal (Rasby, 2007). Early weaning may result in calves having less value at weaning compared to calves weaned at conventional ages (Story et al., 2000). Retaining ownership of young calves through backgrounding can be useful for increasing their value.

Limit-feeding non-lactating cows or cow-calf pairs in confinement can also reduce grazing pressure on pastures, while maintaining cow BCS or BW (Loerch, 1996; Tjardes et al. 1998). Brethour et al. (1990) reported similar BW gains and greater pregnancy rates for non-lactating cows fed in confinement compared with lactating cows grazing native pastures. Limit-feeding non-lactating cows at 1.9% BW achieved acceptable gains in BW, BCS, and rump fat (Waggoner and Jaeger, 2014). Therefore, the objective of our study was to evaluate the performance of beef cows and calves subject to a 56-d early or conventional weaning period in either pasture or drylot environments.

Materials and Methods

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

Animals. Spring-calving Angus-cross cows (n = 167; initial BW = 599 ± 54.5 kg; 5 ± 2.4 yr; initial BCS = 5.5 ± 0.54) and calves (n = 167; initial BW = 204 ± 26.7 kg; 153 ± 15 d of age) originating from the commercial cow-calf herd of the Western Kansas Agricultural Research Center in Hays, KS were used in this study. At approximately 60 d of age, all calves vaccinated against clostridial diseases (Ultrabac® 7; Pfizer Animal Health, Exton, PA) and steers were castrated. At the initiation of the study on August 19, cow-calf pairs were stratified by calf age, cow BW, and cow BCS and assigned randomly to 1 of 4 weaning treatments with 4 pen or pasture replicates/treatment. Treatments were as follows: weaning at 153 d of age followed by 56 d of limit feeding in confinement for both cow and calf (**E-D**), confinement of cow and calf together for a 56-d period of limit feeding followed by weaning at 209 d of age (**C-D**), weaning at 153 d of age followed by a 56-d grazing period for both cow and calf (**E-P**), and a 56-d

[1] Appreciation is expressed to Elanco Animal Health of Greenfield, IN for their support of this research.

grazing period with cow and calf together followed by weaning at 209 d of age (**C-P**).

Cows and calves across all treatments were weighed individually and calves were given initial vaccinations against respiratory pathogens (Bovi-Shield Gold® 5; Pfizer Animal Health, Exton, PA) and clostridial pathogens (Ultrabac® 7; Pfizer Animal Health, Exton, PA). Calves were treated for internal and external parasites (Dectomax® Injectable; Zoetis Inc., Kalamazoo, MI), given injectable trace minerals (Multimin® 90; Multimin USA Inc., Fort Collins, CO), and steers were given a growth-promoting implant (Ralgro®; Intervet Inc., Merck Animal Health, Summit, NJ). Calves were re-vaccinated for viral respiratory pathogens and clostridial pathogens 14 d after study initiation.

Drylot Treatments. Cows and calves assigned to E-D and C-D were placed into the feedlot at the Western Kansas Agricultural Research Center for 56 d. Calves assigned to E-D were separated from their dams and placed in feedlot pens (n = 4, minimum area = 200 m^2 / calf; bunk space = 0.46 m / calf) and afforded *ad libitum* access to water via concrete tanks. Calves were fed a weaning diet (Table 1) formulated to promote a 1-kg ADG at a DMI of 2.5% of BW. Bunks were evaluated each morning at 0630 h, and feed was delivered once daily at 0700 h. Bunks were managed using a slick-bunk management method to minimize feed refusals (Pritchard and Bruns, 2003). If all feed delivered to a pen was consumed, delivery at the next feeding was increased to approximately 102% of the previous delivery. Diet samples were collected from bunks weekly and frozen at -20°C. Samples were composited by weight at the conclusion of the study and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, and ADF (Table 1). Diet NE values were calculated from detergent fiber analyses using equations provided by NRC (2000).

Table 1. Composition of the diet fed to early-weaned calves in confinement

Ingredient composition	% DM
Sorghum silage	21.9
Dry rolled sorghum grain	63.4
Wet distillers grains	6.1
Soybean meal	5.1
Supplement*	3.4
Nutrient composition†	DM basis
CP, % DM	18.1
NE_m, Mcal/kg DM	1.81
NE_g, Mcal/kg DM	1.09

* Supplement contained ammonium sulfate, limestone, urea, salt, Rumensin 90® (300 mg $head^{-1} \cdot d^{-1}$), Tylan 40® (90 mg $head^{-1} \cdot d^{-1}$) , and a trace-mineral premix.
† Nutrient analysis conducted by SDK Laboratories, Hutchison, KS.

Cows assigned to E-D were separated from their calves and placed in earth-floor pens (n = 4, minimum area = 1033 m^2 / cow; linear bunk space = 0.65 m / cow) and afforded *ad libitum* access to water via concrete tanks. Cows were limit fed a roughage-based diet at 1.6% of initial BW (Table 2). Feed was delivered once daily at 0700 h. Diet samples were collected from bunks weekly and frozen (-20°C). Diet samples were composited by weight at the conclusion of the study and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, and ADF (Table 2). Diet NE values were calculated from detergent fiber analyses using equations provided by NRC (2000).

Table 2. Composition of the diet fed to beef cows in confinement

Ingredient composition	% DM
Ground hay*	80.6
Dry rolled sorghum grain	10.4
Wet distillers grains	7.9
Calcium carbonate	0.30
Salt	0.30
Vitamin and mineral premix	0.30
Nutrient composition†	DM basis
CP, % DM	13.2
NE_m, Mcal/kg DM	1.68

* Native prairie hay blended with forage sorghum hay.
† Nutrient analysis conducted by SDK Laboratories, Hutchison, KS.

Cows and calves assigned to C-D were placed as pairs into feedlot pens (n = 4, minimum area = 1033 m^2 / cow; bunk space = 0.65 m / cow) and afforded *ad libitum* access to water via concrete tanks. Cows were limit fed a forage-based diet at 2.0% of initial BW that was formulated to meet nutrient requirements of pregnant cows in late lactation (NRC, 2000). Calves assigned to C-D were offered the same diet fed to E-D (Table 1) at a daily DM allowance of 2.0% of initial BW. Creep panels were used to allow calves undisturbed access to the weaning diet. Cow and calf bunks were evaluated each morning at 0630 h and feed was delivered once daily at 0700 h. Diet samples were collected from bunks weekly and frozen (-20°C). Samples were composited by weight and nutrient composition was analyzed as described above.

Pasture Treatments. Cows and calves assigned to E-P and C-P were placed onto the native pastures at the Western Kansas Agricultural Research Center for 56 d. Predominant forage-plant species were sideoats grama, western wheatgrass, blue grama, Japanese brome, and buffalograss. Calves assigned to E-P were separated from their dams and placed in feedlot pens for 4 d (n = 4, minimum area = 200 m^2 / calf; bunk space = 0.46 m / calf) and afforded *ad libitum* access to water via concrete tanks. Calves were fed native prairie hay *ad libitum*. Hay was delivered once daily at 0700 h. On the afternoon of d 4, calves were released into 1 of 4 assigned pastures. Each pasture (11 ± 0.4 ha) provided continual access to surface water and was stocked at 0.8 ha/calf for 56 d.

Two permanent 100-m transects were established in each pasture at the onset of the study in order to estimate forage quality and above-ground forage biomass. Pasture forage quality and biomass were estimated by clipping all plant material from within randomly-placed sampling

frames (0.25 m^2; n = 10 / pasture) at a height of 1 cm on 8/19, 9/16, and 10/14. Range forage samples were dried in a forced-air oven (50 °C; 96 h) and weighed to estimate biomass availability. Samples were subsequently composited by sampling date on an equal-weight basis at the conclusion of the experiment and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, and ADF (Table 3).

Table 3. Nutrient composition of range forage grazed by cows and calves

Sampling Date	CP, % DM	NDF, % DM	ADF, % DM
Calves - early weaned			
08/19/2014	6.8	71.1	46.2
09/16/2014	5.9	76.2	51.2
10/14/2014	5.5	74.9	51.6
Cows - early weaned			
08/19/2014	6.2	71.6	45.8
09/16/2014	5.5	76.7	51.1
10/14/2014	4.6	77.2	52.4
Cow-calf pairs - conventionally weaned			
08/19/2014	5.8	70.4	44.6
09/16/2014	5.2	74.9	49.3
10/14/2014	5.4	75.1	50.5

Cows assigned to E-P were separated from their calves and placed in feedlot pens for 4 d (n = 4, minimum area = 1033 m^2 / cow; bunk space = 0.65 m / cow) and afforded *ad libitum* access to water via concrete tanks. Cows were fed the same prairie hay offered to E-P calves for *ad libitum* intake during this period. Hay was delivered once daily at 0700 h. Cows were released into assigned pastures on the afternoon of d 4 and remained there 56 d. Each pasture (n = 4, 15 ± 0.4 ha) was stocked at 1.2 ha/cow and provided continual access to surface water. Pasture forage quality (Table 3) and total forage biomass (Table 4) were collected as described above on 8/19, 9/16, and 10/14.

Cows and calves assigned to C-P were placed as pairs directly onto native range pasture (n = 4, 15 ± 0.4 ha) for 56 d. Pastures were stocked at 1.6 ha/pair and provided continual access to surface water. Pasture forage quality (Table 3) and total forage biomass (Table 4) were collected as described above on 8/19, 9/16, and 10/14.

Final Phase. Following the 56-d study period, cows and calves were individually weighed. Animals assigned to E-P and C-P were transported to the Western Kansas Agricultural Research Center feedlot. Cows and calves assigned to C-P and C-D were separated at that time and assigned to a new pen (n = 4/treatment for cows, 4/treatment for calves). To equalize gut-fill between treatments, all calves were fed a common diet (Table 1) at 2.0% of BW for 7 d and all cows were fed a common diet (Table 2) at 1.6% of BW for 7 d.

Data Collection. Calf BW were individually measured on d 0, 28, 56, and 63. Cows were weighed individually on d 0 and 63. Cows and calves were weighed at 0600 prior to feed delivery. Cow BCS were assigned by two trained observers using a 9-point scale (1 = emaciated, 9 = obese; Wagner et al., 1988) on d 0 and 63. Also on d 0 and 63, rump fat thickness of cows was measured ultrasonically at the midpoint between the hip bone and pin bone 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). Cattle Performance Enhancement Company (**CPEC**, Oakley, KS) software was used to collect ultrasound images. Rump fat thickness was estimated with procedures that incorporated image analysis software integral to the CPEC software (Brethour, 1994).

Statistical Analysis. Cow and calf performance were analyzed as a mixed model with a 1-way treatment structure in factorial arrangement of a completely-randomized design (PROC MIXED; SAS Inst. Inc., Cary, NC). Pen or pasture was the experimental unit. Class factors included pen or pasture, weaning treatment, and weaning diet. The model statement included terms for the fixed effects of weaning treatment, weaning diet, and weaning treatment × weaning diet.

Native range biomass data were analyzed as a mixed model with a 1-way treatment structure in a completely-randomized design (PROC MIXED; SAS Inst. Inc., Cary, NC). Pasture was the experimental unit. Class factors included treatment and pasture. The model statement included a term for the fixed effect of treatment only.

When protected by a significant *F*-test ($P < 0.05$), Least Squares treatment means were separated using the method of Least Significant Difference. Means were considered different when $P \leq 0.05$.

Results and Discussion

Forage Biomass. Available pasture forage biomass was greater ($P \leq 0.01$) for E-P calves than for either E-P cows or C-P cow-calf pairs for the duration of our study (Table 4). This was expected because of lesser grazing pressure afforded by calves compared with either cows or cow-calf pairs. There were no differences ($P \geq 0.21$) in available forage biomass were between C-P cow-calf pairs or E-P cows at any time during our study. Range-forage biomass declined in quantity throughout the study in all treatments.

Calf Performance. Calf BW was not different ($P \geq 0.06$) between treatments at the beginning of the study or on d 28 (Table 4). On d 63, there was an interaction ($P = 0.05$) between diet and weaning treatment. Calves managed in confinement, both weaned and non-weaned, had greater BW than calves managed on pasture. Calves suckling their dams had greater BW than weaned, unsupplemented calves grazing native pastures. Average daily gains were influenced also by diet and weaning treatments (diet × weaning – $P \leq 0.03$). In general, calves managed in confinement and fed concentrate-based diets (i.e., E-D and C-D) had greater ADG than unsupplemented calves maintained on pasture (i.e., E-P and C-P). Weaned calves on pasture had lesser ($P < 0.01$) ADG than suckling calves on pasture from d 0 to 28 and from d 0 to 63.

Cow Performance. Cow BW, BCS, and rump-fat thickness were not different ($P \geq 0.36$) between treatments at the beginning of the study (Table 5). Cow BW on d 63 was greatest ($P < 0.01$) for non-lactating cows on pasture,

intermediate for non-lactating cows fed in confinement and least for cows that continued to suckle calves. Overall BW change was influenced by both diet and weaning status (diet × weaning – $P = 0.05$). Non-lactating cows maintained on pasture had lesser BW loss than other treatments; BW loss by confined, non-lactating cows and lactating cows maintained on pasture was lesser than that by confined lactating cows. Cow BCS on d 63 and BCS change from d 0 to 63 were influenced ($P < 0.01$) by diet and weaning status. Non-lactating cows fed in confinement had lesser BCS on d 63 and greater BCS loss from d 0 to 63 than all other treatments.

Trends in BW and BCS may be interpreted to indicate that DMI of the cows assigned to the E-C treatment was not adequate to maintain BW or BCS; however, rump-fat data do not support this conclusion. Rump-fat depth on d 63 was greater ($P < 0.01$) for non-lactating cows maintained on pasture than for lactating cows in either pasture or drylot environments; non-lactating cows in confinement were intermediate to and not different from these treatments (Table 5). Similarly, change in rump-fat depth was greatest (diet × weaning - $P < 0.01$) for non-lactating cows on pasture and least for lactating cows in either pasture or drylot environments. Non-lactating cows maintained in confinement were intermediate to and different from these treatments.

Implications

Results were interpreted to indicate that early weaning spared cow BW and rump fat compared to weaning at conventional calf ages. Performance of cows was acceptable when either limit-fed under drylot conditions or maintained in a pasture environment. Conversely, calf performance was generally greater in confinement that on pasture.

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Table 4. Forage biomass available (kg forage DM/ 100 kg BW) to weaned calves, non-lactating cows, and cow-calf pairs during a 56-d grazing period

Item	Weaned calves[*]	Non-lactating cows[†]	Cow-calf pairs[‡]	SEM
08/19/2014, kg	812.3[a]	443.3[b]	356.2[b]	65.65
09/16/2014, kg	806.5[a]	389.8[b]	317.9[b]	54.04
10/14/2014, kg	661.1[a]	345.2[b]	345.2[b]	49.70

[*]Calves were early weaned in a pasture environment and not supplemented for 56 d (4 pastures; 12 or 13 calves/pasture).
[†]Dams of early-weaned calves in a pasture environment and not supplemented for 56 d (4 pastures; 12 or 13 cows/pasture).
[‡]Cow-calf pairs grazed together in a pasture environment and not supplemented for 56 d (4 pastures; 8 or 9 pairs/pasture).
[a, b]Within a row, means without a common superscript differ ($P \leq 0.01$).

Table 5. Performance of beef calves that were weaned early or paired with dams in either confinement or pasture environments

						P-value		
Item	Weaned calves - confined[*]	Non-weaned calves - confined[†]	Weaned calves - pasture[‡]	Non-weaned calves - pasture[§]	SEM	Diet	Weaning	Diet x Weaning
Initial BW, kg	208	205	207	204	4.1	0.83	0.50	0.99
d 28 BW, kg	242	244	227	243	4.6	0.07	0.06	0.16
d 63 BW, kg	277[c]	285[c]	226[a]	254[b]	5.0	< 0.01	< 0.01	0.05
ADG d 0 to 28, kg	1.2[c]	1.4[b]	0.7[a]	1.4[b]	0.05	< 0.01	< 0.01	< 0.01
ADG d 28 to 63, kg	1.0[b]	1.2[c]	-0.3[a]	0.3[a]	0.04	< 0.01	< 0.01	0.03
ADG d 0 to 63, kg	1.1[c]	1.3[d]	0.3[a]	0.8[b]	0.04	< 0.01	< 0.01	< 0.01

[*] Calves were weaned in a drylot environment and fed a growing diet 56 d (4 pens; 8 or 9 calves/pen).
[†] Cow-calf pairs confined together in a drylot environment fed complete diets for 56 d (4 pens; 8 or 9 pairs/pen).
[‡] Calves were weaned in a pasture environment and not supplemented for 56 d (4 pastures; 12 or 13 calves/pasture).
[§] Cow-calf pairs grazed together in a pasture environment and were not supplemented for 56 d (4 pastures; 12 or 13 pairs/pasture).
[a, b, c, d] Within a row, means without a common superscript differ ($P \leq 0.01$)

Table 5. Performance of pregnant beef cows in confinement and pasture environments either post-weaning or while suckling calves

						P-value		
Item	Post-weaning - confined[*]	Suckling - confined[†]	Post-weaning - pasture[‡]	Suckling - pasture[§]	SEM	Diet	Weaning	Diet x Weaning
BW, kg								
d 0	613	603	597	603	8.6	0.37	0.85	0.36
d 63	583[b]	555[a]	596[c]	570[ab]	8.5	< 0.01	< 0.01	0.93
Change, d 0 to 63	-30.0[b]	-48.4[c]	-1.0[a]	-33.7[b]	3.58	< 0.01	< 0.01	0.05
BCS								
d 0	5.5	5.4	5.5	5.5	0.08	0.56	0.78	0.47
d 63	4.5[a]	5.0[b]	5.1[b]	5.0[b]	0.07	< 0.01	< 0.01	< 0.01
Change, d 0 to 63	-1.0[a]	-0.4[b]	-0.4[b]	-0.6[b]	0.70	< 0.01	< 0.01	< 0.01
Rump fat depth, mm								
d 0	5.43	5.67	4.91	5.44	0.054	0.49	0.48	0.78
d 63	6.69[ab]	6.05[a]	8.33[b]	5.89[a]	0.057	0.19	< 0.01	0.12
Change, d 0 to 63	1.262[b]	0.393[c]	3.411[a]	0.449[c]	0.030	< 0.01	< 0.01	< 0.01

[*] Cows were maintained in a drylot environment and fed a forage-based diet 56 d (4 pens; 8 or 9 cows/pen).
[†] Cow-calf pairs confined together in a drylot environment fed complete diets for 56 d (4 pens; 8 or 9 pairs/pen).
[‡] Cows were maintained in a pasture environment and not supplemented for 56 d (4 pastures; 12 or 13 calves/pasture).
[§] Cow-calf pairs grazed together in a pasture environment and were not supplemented for 56 d (4 pastures; 12 or 13 pairs/pasture).
[a,b,c,d] Within a row, means without a common superscript differ ($P \leq 0.01$)

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Effects of Octacosanol on Non-Seasonal Spermatogenesis in Ovine

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ABSTRACT: This study was conducted to understand the benefits of utilizing octacosanol as an additive to increase fertility of rams during the non-seasonal time of the year. Rams of Suffolk and Rambouillet influence were placed on a 60-d trial to determine the value of octocasanol as a feed supplement to promote semen production through the summer months. Rams were randomly divided into two groups of mixed breeds. Treatment group was fed a balanced ration containing 0.25% octocasanol per ton, while control rams were fed identical ration with no added octocasanol. Rams were fed at the rate of 6 lbs a day with alfalfa hay supplementation three times a week. Final collection was June 27 when ambient stress is generally at its highest point. Semen characteristics such as scrotal circumference, volume, concentration, and motility were used in the evaluation of the success of the product. Scrotal circumference (SC) measurements taken at onset of project to final showed no significant differences among control or treatment (P=0.21) respectively although, the treatment group saw a larger increase in SC when compared to control (0.81±.01, 0.31±.01) respectively. Volume of ejaculate of both treatment and control also showed no significant difference from initial to final collection (P=0.13) although as SC in the middle of summer the treatment group did show some increase when compared to control (0.32±.01, -0.60±.01 respectively). The same progressions were observed with concentration and motility of ejaculates from beginning to final collection although again there was no significance (P=0.51, P=0.34 respectively). Concentration of ejaculate from treatment, again showed some increase from the control rams (2.32 x 10^9, -1.28 x 10^9 respectively). Like the previous measurements, motility also showed an increase from the treatment group when compared to the control rams (0.31 x 10^9, -0.27 x 10^9 respectively). Rams fed octacosanol for 60 d tended to have larger scrotal circumference, produced larger ejaculates with greater volume and sperm concentrations than control rams during the harshest period of the year for these parameters.

Keywords: Octacosanol, spermatogenesis, ovine

INTRODUCTION

With an ever-increasing demand for food protein in our world's growing population, the need for small ruminant products to meet growing markets is becoming extremely important. The best way to initially maximize livestock production is to be able to be reproductively efficient. Most small ruminants, however, are typically short day breeders. Most ewes maximize reproductive efficiency when breeding occurs in the fall, followed by lambing in the spring. One of the problems that most producers face now is the demand for fall born lambs. The main complication with being able to raise fall born lambs comes with reproductive inefficiency in the ram. This is especially prevalent in Suffolk or Hampshire rams, but across all breeds, over exposure to ambient temperature negatively effects fertility and conception rates during the late spring and early summer (Hulet et al. 1956). However, it has been noted that any increase in nutritional quality will improve fertility in both the male and female. Due to an inability to be able to control environmental conditions or manipulate specific photo-periods in a commercial sheep setting, nutrition and stress limitations are the only external factors that can be controlled to help aid in and increase in sperm quality. Wheat germ oil has proven to aid in reproductive function in different species of livestock. Bonadonna and Kaan (1954) administered fresh wheat germ oil to bulls and it resulted in a larger ejaculate with better quality semen with more viability. Octacosanol is extracted from wheat germ oil and is readily available in powder form and is easily utilized in feed rations. Little work has been conducted to determine the effects of octacosanol on reproductive parameters in sheep.

MATERIALS AND METHODS

Thirty mature Rambouillet and Suffolk rams were randomly selected for a feeding trial that began on February 6th, 2012 at the Angelo State Management Instruction and Research (MIR) Center in San Angelo, Texas. All rams were brought in from a pasture setting

45 km from the test location, and started on a full feed pre-conditioning phase in a pen setting for approximately 60 days. The rams were divided into 4 separate pens (18.6 m^2/ram) to allow for adequate room and closer inspection of health and wellness. The rams were fed a ration of six pounds a day/head and supplemented with alfalfa hay three times a week and had clean fresh water and shade available ad libitum. At the completion of the pre-condition phase, all rams were individually stimulated via electro-ejaculation, collected, and evaluated. Volume, concentration, and a motility score were given, and Scrotal Circumference was measured and recorded. Breed type was also recorded and used as a factor in analysis. At the completion of the pre-conditioning phase and initial semen collection, the rams were randomly assigned to either a control or treatment group; where the rams were put on the same feed rations with the treatment group ration containing octacosanol (Table 1).

Table 1. Ingredient and nutrient composition (DM basis) of Control ration and Treatment ration

	Control Diet %	Treatment Diet %
Ingredient		
Yellow Corn	24.51	24.51
Dehydrated Alfalfa	22.51	22.51
Cottonseed Hulls	20.01	20.01
Soybean Meal	11.50	11.50
Wheat Middlings	5.00	5.00
Corn Chops	5.00	5.00
Cane Molasses	4.00	4.00
Corn Gluten Meal	1.25	1.25
Fish Meal	1.25	1.25
Octacosanol	0.00	0.25
Nutrient Composition		
DM	89.936	
Crude Protein	16.997	
Crude Fat	2.901	
Crude Fiber	15.883	
TDN	64.74	

[1]Premix: 1% NaCl, 1.08% Limestone, 0.6% NH_4Cl, 0.06% Vitamin A, 0.02% Vitamin E, 0.01 Vitamin

Eleven rams were put into the control group, while 19 rams were placed into the treatment group and started on feed. They were fed six pounds a day per animal, and provided alfalfa hay three times a week ad libitum. The trial phase lasted sixty days and the rams were then collected through electro-ejaculation, and semen measurements were recorded. Volume, concentration, motility score, and scrotal circumference were analyzed using the GLM procedures of SAS (Cary, NC) with each individual animal being identified separately and compared between treatment and breed type. Treatments will be considered different when alpha is 0.05 or less.

RESULTS AND DISCUSSION

Breed influences played a role in initial differences as well as change in scrotal circumference (SC), concentration (Con.), volume (Vol.), and motility (Mot.) The Suffolk rams had a smaller SC ($P < 0.05$), but were similar in terms of Con., Vol., and Mot. ($P > 0.05$). Similarly when comparing initial values from control to the treatment group the values from the initial collection were similar ($P > 0.05$).

Scrotal Circumference. The comparison of initial SC to final SC in Table 2 shows the increase in cm. of the increase in SC of the treatment group. While it is not significant ($P > 0.05$), it is important to note the difference from initial to final and the added performance of the treament group. The change in the treatment group ($0.81x10^9$) shows the added performance from the higher plane of nutrition that the supplement provided. Martinez-Velasquez (2003) state through their experiment that scrotal circumference is directly related to fertility, and even though Table 2 does not represent a significant P-Value ($P > 0.05$) it is still valuable to show the increase in size (cm.) and ultimately fertility.

Volume. Table 2 specifically analyzes the volume of semen in mL and the results show that the rams from the control group had a greater initial volume ($3.66 x10^9$ mL) than those of the treatment group ($2.92 x 10^9$ mL). Fuquay (1981) shows the effects of heat stress and fertility and the relevance of that study to this project is evident in the fact that not only did the treatment group have an increase in semen volume, but the fact that the final collection was made in the middle of the summer months (June 27th) had an adverse affect and decreased actual average volume from the control group. While understanding the ambient affect that Fuquay (1981) explains in his experimental procedures, it is evident that the heat stress brought about by the environment played a negative effect on the control group. Alternatively, even though there was no significance in the final volume ($P > 0.05$) the ability of the treatment group to increase the change in the volume on average .32 mL, and that it maintained and did not show the negative effects of ambient temperature like the control group.

Concentration. Initial concentration levels between the treatment and control groups were similar ($P > 0.05$). By analyzing the final concentration and the change in concentration from Table 3 the results reveal a statistical increase in concentration for the treatment group. While the data is not considered significant ($P > 0.05$), it is similar, like volume, that the treatment group did not suffer from the ambient effect of heat stress like the control group. The control group shows a negative relation with ambient effect due to the decrease in final concentration on the June 27th collection date. Again, these numbers do not match up to be significantly representative because of its P-Value, yet it still shows the treatment groups ability to better maintain reproductive function through adverse

summer conditions than those rams delegated to normal forms of supplementation without octacosanol.

Motility. Similar to volume and concentration, motility at the original collection date was similar in both the control and treatment groups ($P > 0.05$). Goerke et al. (1970) makes the statement in his research that a higher percent motility relates to a greater correlation of percent normal sperm in Southdown rams, which would correlate to higher conception rates to ewes' exposed. There was no statistical significant difference at the final ejaculation ($P > 0.05$), but the fact that there was an increase in motility score of the treatment group versus the decline of the control group indicates some substantial effects to the supplement. Though not significantly represented through the data, the added effects of the treatment through the non- seasonal ambient conditions allows for maintained or increased fertility versus conventional supplementation.

IMPLICATIONS

The rams used within this performance, though not showing statistical significant changes in semen quality, did trend to be affected by the supplementation of octacosanol. While considering the ambient affect that limits high performing semen production, the rams treated with octacosanol not only maintained consistent levels through the middle of the summer months, but also improved slightly as compared to those subject to the control. All four measurements of semen quality showed an increase from the initial to final collection, and because breeds were allocated randomly to each treatment group, effects were consistent across the board. Though it is necessary for increased research on the product, the positive trend and correlation to octacosanol treatment shows that there may be benefits to supplementing this product when there is a need for non-seasonal, ovine reproductive services.

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Table 2. Initial, Final, and Change in Scrotal Circumference of both treatment and control groups

	Control	Treatment	Standard Error	P-Value
Scrotal Circumference, cm. (Initial)	33.09 ± .01	32.57 ± .01	0.92 ± .01	0.6636
Scrotal Circumference, cm. (Final)	33.40 ± .01	33.39 ± .01	0.98 ± .01	0.9909
Scrotal Circumference, cm. (Change in SC)	0.31 ± .01	0.81 ± .01	0.31 ± .01	0.2130

Table 3. Initial, Final, and Change in Volume of both treatment and control groups

	Control	Treatment	Standard Error	P-Value
Initial Volume, ml	3.66 ± .01	2.92 ± .01	0.31 ± .01	0.0709
Final Volume, ml	3.06 ± .01	3.24 ± .01	0.33 ± .01	0.6636
Change in Volume, ml	-0.60 ± .01	0.32 ± .01	0.46 ± .01	0.1272

Table 4. Initial, Final, and Change in Concentration of both treatment and control groups

	Control	Treatment	Standard Error	P-Value
Initial Concentration	2.50×10^9	1.94×10^9	2.78×10^8	0.1195
Final Concentration	2.37×10^9	2.17×10^9	2.68×10^8	0.5573
Change in Concentration	-1.28×10^9	2.32×10^9	4.34×10^8	0.5135

Table 5. Initial, Final, and Change in Motility of both treatment and control groups

	Control	Treatment	Standard Error	P-Value
Initial Motility	4.45×10^9	4.05×10^9	0.37±.01	0.4029
Final Motility	4.18×10^9	4.36×10^9	0.30±.01	0.6265
Change in Motility	-0.27×10^9	0.31×10^9	0.48±.01	0.3419

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EFFECTS OF DRY AND WET CONDITIONS DURING THE PRE-WEANING PHASE ON SUBSEQUENT FEEDLOT PERFORMANCE AND CARCASS COMPOSITION OF BEEF CATTLE

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ABSTRACT: The objective of this study was to determine the effects of dry and wet conditions during the pre-weaning phase of beef cattle production on subsequent feedlot performance and carcass characteristics. Steers (n = 7,439) and heifers (n = 2,380) finished in 16 feedlots in southwestern Iowa through the Tri-County Steer Carcass Futurity Cooperative (Lewis, IA) were used for a retrospective analysis. Cattle originated in the Midwest, were born in February, March, or April, and were slaughtered between 2003 and 2014. Feedlot performance and carcass composition data were obtained for each animal. Palmer Drought Severity Index (PDSI) values were obtained for each animal for the pre-weaning forage growing season on a monthly basis. These values were used to classify conditions as dry (mean PDSI value $\leq$ -2.00), normal (mean PDSI value > -2.00 and < 2.00), or wet (mean PDSI value $\geq$ 2.00) for the cool season, warm season, and combined seasons. Mixed models were used to evaluate the effects of dry and wet conditions on subsequent performance. Birth year, feedlot, and sex were included as fixed effects. Average daily gain was greater ($P < 0.03$) for cattle from the dry class than those from the wet class during the cool season and the combined seasons. Cattle from the dry and normal classes for both the cool season and combined seasons had greater ($P < 0.02$) final BW than those from the wet class. During the cool season, HCW was greater ($P < 0.0001$) for the normal class than wet class, although HCW was greater ($P < 0.04$) for the dry class compared with normal and wet during the combined seasons. Calculated yield grade was improved ($P < 0.01$) for the normal class during the cool season compared with the dry and wet classes. For both the warm and combined seasons, the dry class had improved ($P < 0.02$) calculated yield grade compared with normal and wet classes. For the cool season, the dry and normal classes had greater ($P < 0.03$) marbling scores than the wet class. For marbling score in the warm season, the normal and wet classes were greater ($P < 0.02$) than the dry class. In conclusion, this study indicates that both dry and wet conditions during the pre-weaning phase may impact ultimate feedlot performance and carcass composition.

Key words: carcass, drought, feedlot

INTRODUCTION

Drought has been a scourge to cattle producers for thousands of years. In spite of the many advances made in production practices, beef production around the world remains heavily dependent on adequate rainfall to provide for forage growth. The inability to directly alleviate drought increases the need to understand its negative effects on production and to seek to minimize them through best management practices.

To meaningfully interpret the effects of drought on beef cattle production, an appropriate measure of drought must be utilized. The Palmer Drought Severity Index (PDSI) is a prominent and widely applied drought index. A PDSI value is calculated for each month within each climate division based on a calculation outlined by Palmer (1965), which includes measures of precipitation, evapotranspiration, soil water holding capacity, and runoff. There are generally 6 to 10 climate divisions per state. Palmer Drought Severity Index values typically fall in the range of -4 to 4 (with a theoretical range of -10 to 10), with -4 indicating extreme drought and 4 indicating extreme moisture surplus. The PDSI is an intermediate range drought measure. It calculates the effects of abnormally dry or wet preceding months on current moisture availability, but is also fairly responsive to new moisture changes. This makes it appropriate for describing moisture surplus or deficit as is likely to influence forage growth and forage-dependent livestock production.

Dry or wet conditions during the pre-weaning phase can impact growing animals through both direct and maternal dietary effects (Neville, 1962). These effects are most commonly quantified as a change in calf weaning weight, but likely persist post-weaning. Pre-weaning growth rate can affect post-weaning body weight gains and ultimately shift carcass composition (Neville et al., 1962; Café et al., 2009). These studies and others have illustrated specific impacts of pre-weaning nutrient restriction on beef cattle performance in experimental settings. More comprehensive studies of the longitudinal effects of dry and wet conditions on diverse groups of cattle in commercial production settings are limited at best. Anecdotal reports often indicate poor feedlot performance for drought-stressed calves, but this has not been quantified to our knowledge. The objective of this study was to determine the effects of dry and wet conditions during the pre-weaning phase of beef cattle production on subsequent feedlot performance and carcass composition.

MATERIALS AND METHODS

A retrospective analysis was performed using feedlot and carcass data for 7,439 steers and 2,380 heifers. These cattle originated from Iowa (n=6,044), Missouri (n=2,775), Indiana (n=916), Illinois (n=56), and Minnesota

(n=28). They were sourced from 283 producers and were fed through slaughter in 16 feedlots in southwestern Iowa as part of the Tri-County Steer Carcass Futurity Cooperative (**TCSCF**; Lewis, Iowa) between 2003 and 2014 (ranging from 356 to 1,590 per year). All animals used in the present analysis were born in the months of February (n=1,968), March (n=5,145), or April (n=2,706), as reported by producers of origin. All calves were weaned according to individual cow-calf producer practices prior to being transported to a TCSCF feedlot. Cattle were fed and data were collected as described by Reinhardt and Busby (2014). Pens of cattle were slaughtered in 2 groups 28 days apart, with the goal of slaughtering cattle with an average backfat thickness of 1.14 cm. Body weight measurements of cattle were taken individually by TCSCF personnel upon feedlot arrival and immediately prior to shipment of cattle to the slaughter facility. Hot carcass weight was recorded at the slaughter facility, and quality grade was assigned by USDA personnel. Trained employees of the TCSCF measured and recorded individual marbling score, 12^{th} rib fat thickness, LM area, and calculated yield grade (Table 1).

The data set obtained from TCSCF included cow-calf producer zip code for each animal, which was used to assign each animal to the proper climate division within its respective state. Historical PDSI data were obtained from the National Oceanic and Atmospheric Administration (Washington, DC). These data included a numerical PDSI value for each climate region by month. These monthly PDSI values were then assigned to individual animals for each month of the pre-weaning (for all calves) forage growing season (April through August) for their year of birth based on birth date and zip code of origin.

A random sample of PDSI values were individually checked to validate accuracy. Further, each zip code was individually verified to have been assigned to the correct climate district. Following verification, mean PDSI values were calculated for individual animals for 3 time periods: cool forage growing season (April and May), warm forage growing season (June, July, and August), and combined seasons (April through August). For each time period, mean PDSI value was used to assign individual animals to 1 of 3 PDSI drought classes: Dry (mean PDSI value $\leq$ -2.00), Normal (mean PDSI value > -2.00 and < 2.00), or Wet (mean PDSI value $\geq$ 2.00). These classes (Table 2) were the basis for further analysis.

PROC MIXED in SAS 9.4 (SAS Inst., Inc., Cary, NC) was used to analyze data. Feedlot, calf sex, birth year, and drought class were included as fixed effects in the model for each forage growing season. Individual animal was the experimental unit. Least square means were separated by least significant difference when $P \leq 0.05$.

RESULTS

Feedlot Performance

Effects of PDSI drought class on feedlot performance are summarized in Table 3. Drought class during the cool season, warm season, and combined seasons affected (P <0.0001) age on feedlot arrival, feedlot delivery weight, days on feed, and age at slaughter. Drought class during the cool season and combined seasons had an effect ($P \leq 0.05$) on ADG and final weight, but drought class during the warm season did not ($P \geq 0.11$).

Age on feedlot arrival was less ($P < 0.0001$) for cattle in the wet and dry classes when compared with the normal class for the cool season. For both the warm season and the combined seasons, age on arrival was greatest ($P < 0.0001$) for cattle from the dry class and least ($P < 0.02$) for cattle from the wet class. Feedlot delivery weight paralleled age on arrival. For the cool season, cattle from the dry class were the lightest ($P < 0.0001$) on arrival and those from the wet class were the heaviest ($P < 0.01$). Conversely, for both the warm season and the combined seasons, cattle from the dry class were the heaviest ($P < 0.0001$) and those from the wet class were the lightest ($P < 0.0001$).

Average daily gain was greater ($P < 0.0001$) for cattle from the dry class than those from normal or wet during the cool season and greater ($P < 0.01$) than wet during the combined seasons. Cattle from the dry and normal classes for both the cool season and combined seasons had greater ($P < 0.02$) final BW prior to slaughter than those from the wet class.

Cattle from the dry class during the cool season had a greater ($P < 0.0001$) number of days on feed than those from the normal and wet classes and were older at slaughter ($P < 0.03$) than the wet class. For both the warm season and the combined seasons, cattle in the dry class spent the fewest ($P < 0.002$) days on feed and those in the wet class spent the most ($P < 0.002$) days on feed. Age at slaughter was greater ($P < 0.0001$) for cattle in the dry class than for those in the wet class for both the warm season and the combined seasons. The normal class was intermediate for age at slaughter in the combined seasons ($P < 0.0001$), but did not differ from wet in the warm season ($P = 0.59$).

Carcass Composition

Effects of PDSI drought class on carcass composition are summarized in Table 4. Drought class during the cool season and combined seasons had an impact ($P \leq 0.03$) on hot carcass weight (HCW), but during the warm season alone it did not ($P = 0.12$). Dressing percent was not affected ($P = 0.42$) by drought class. Drought class during the cool season, warm season, and combined seasons impacted ($P \leq 0.05$) backfat, LM area, calculated yield grade, and marbling score.

Cattle in the normal class for the cool season had greater ($P < 0.0001$) HCW than those in the wet class. Cattle in the dry class for the combined seasons had greater ($P < 0.04$) HCW than the normal and wet classes.

For the cool season, cattle from the wet class had greater ($P < 0.0001$) backfat depth than those from the normal class. Backfat depth was greater ($P < 0.02$) for cattle from the normal and wet classes compared with the dry class for both the warm season and the combined seasons. Loin muscle area was greater ($P < 0.01$) for the normal class during the cool season. For the warm season, LM area was greater ($P < 0.01$) for the dry class than the normal class. The dry class had greater ($P < 0.01$) LM area than normal and wet classes for the combined seasons. Calculated yield grade was improved ($P < 0.01$) for the normal class compared with the dry and wet classes, during the cool season. During the warm and combined seasons, the dry

class had improved ($P < 0.02$) calculated yield grades compared with normal and wet classes.

For the cool season, the dry and normal classes had greater ($P < 0.03$) marbling scores than the wet class. For the warm season, the normal and wet classes had greater ($P < 0.02$) marbling scores than the dry class. The dry class did not differ ($P > 0.05$) from either the normal class or the wet class during the combined seasons, although marbling score was greater ($P < 0.02$) for the normal class than the wet class.

DISCUSSION

Many of the effects of dry or wet conditions during the pre-weaning phase assessed in this study, while significant, are relatively small in magnitude. Nevertheless, they illustrate that overall plane of nutrition before weaning, as affected by precipitation, may induce physiological responses that remain measurable in the feedlot and post-slaughter. Additionally, these responses do not appear to always be negative in nature, as post-weaning production improved for calves from dry pre-weaning conditions in several cases.

Forage yield is broadly accepted to be decreased by drought, but forage and diet quality responses to drought are more complex. While Peterson et al. (1999) and Craine et al. (2009) showed improved forage quality during drought conditions, this may not be representative of negative changes in diet quality related to reduced plant growth (Laude, 1953). A reduction in overall diet quality may reduce calf weaning weight and maternal milk yield (Neville, 1962). The impacts of pre-weaning nutritional restrictions can continue much later in an animal's life. When fed under the same conditions in the finishing phase, Café and colleagues (2009) observed that calves that had been restricted to slow growth during the pre-weaning phase had reduced final BW when compared to calves that had not been nutritionally restricted pre-weaning.

Although poor pre-weaning nutritional planes are generally attributed more to dry than wet conditions, our data indicate that wet conditions pre-weaning may also impact subsequent calf performance. Effects seem to be negative when wet conditions occur during the cool season. This is likely a function of decreased nutrient density in the diet as plant dry matter content falls.

Dry or wet conditions during the cool season appear to affect calves differently than the same conditions during the warm season. This likely reflects both calf stage of development and diet composition. During the cool season, most of the calf's diet is milk. While dry or wet conditions can certainly impact maternal milk yield, the differences in forage quality and availability likely have a less direct impact on calves at this stage. Conversely, calves consume increasing amounts of forage as they age, which fell during the warm season in this study. Any moisture-induced changes in forage quality or availability during the warm season can therefore have a direct impact on the calf's plane of nutrition while also impacting milk production.

Management responses to dry or wet conditions are also likely to alter calf physiological response. Early weaning is a common management response to drought conditions, but does not appear to have been utilized heavily by producers in the current study. Additionally, producers may be more likely to creep feed calves during periods of drought than at other times. This has the potential to improve the overall plane of nutrition enough to increase rate of gain pre-weaning and mitigate or even reverse the effects of drought on calf performance.

In conclusion, these data highlight that not only drought, but also wet conditions, observed during the pre-weaning period in spring-calving, Midwestern beef herds may have economically relevant impacts on feedlot performance and carcass composition. Further research is warranted to determine the effects of pre-weaning precipitation on calf health, as well as the impacts of pre-weaning precipitation on post-weaning calf performance in other U.S. regions.

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Table 1. Feedlot and carcass characteristics of a subset of cattle finished through the Tri-County Steer Carcass Futurity

Item	n	Mean	SD	Minimum	Maximum
Age on feedlot arrival, d	9,790	245	48.9	126	954
Days on feed	9,819	174	32.2	4	252
Age at slaughter, d	9,544	418	37.4	277	882
Feedlot delivery weight, kg	9,819	280	51.1	118	504
ADG, kg/d	9,642	1.48	0.250	-0.19	2.49
Final body weight, kg	9,647	537	54.2	218	738
HCW, kg	9,572	331	33.2	218	450
Dressing percent, %	9,572	61.5	1.69	47.6	71.8
Calculated yield grade	9,572	2.93	0.570	0.11	5.39
Fat depth, cm	9,572	1.18	0.343	0.05	3.30
LM area, cm^2	9,572	79.7	7.42	52.9	125.8
Marbling score[1]	9,572	1,033	75.2	800	1,480

[1] $Trace^0$=800 $Slight^0$=900 $Small^0$=1000 $Modest^0$=1100 $Moderate^0$=1200 $Slightly\ Abundant^0$=1300 $Moderately\ Abundant^0$=1400.

Table 2. Number of cattle in each drought class[1] for each growing season period[2]

	PDSI Drought Classes		
Growing Season Period	Dry, n	Normal, n	Wet, n
Cool season	823	4,365	4,631
Warm season	1,711	4,040	4,068
Combined seasons	1,711	4,040	4,068

[1] Dry = Mean PDSI ≤ -2.00, Normal = Mean PDSI > -2.00 and < 2.00, Wet = Mean PDSI ≥ 2.00.
[2] Cool season = April and May, Warm season = June through August, Combined seasons = April through August.

Table 3. Effects of drought class[1] for each growing season period[2] on feedlot performance

	PDSI Drought Classes[1]			
Item	Dry	Normal	Wet	*P*-value
Age on feedlot arrival, d				
Cool season	236 ± 2.4^b	251 ± 1.2^a	241 ± 1.4^b	<0.0001
Warm season	258 ± 1.9^a	244 ± 1.1^b	240 ± 1.4^c	<0.0001
Combined seasons	265 ± 1.9^a	249 ± 1.2^b	234 ± 1.4^c	<0.0001
Feedlot delivery BW, kg				
Cool season	252 ± 2.6^c	279 ± 1.3^a	273 ± 1.5^b	<0.0001
Warm season	280 ± 2.1^a	274 ± 1.2^b	269 ± 1.5^c	<0.0001
Combined seasons	284 ± 2.1^a	276 ± 1.3^b	266 ± 1.5^c	<0.0001
ADG, kg				
Cool season	1.46 ± 0.012^a	1.41 ± 0.06^b	1.40 ± 0.007^b	<0.0001
Warm season	1.40 ± 0.010	1.41 ± 0.06	1.41 ± 0.007	0.77
Combined seasons	1.43 ± 0.010^a	1.42 ± 0.06^{ab}	1.40 ± 0.007^b	0.03
Final BW, kg				
Cool season	521 ± 2.6^a	522 ± 1.3^a	513 ± 1.5^b	<0.0001
Warm season	514 ± 2.1	517 ± 1.2	520 ± 1.5	0.11
Combined seasons	522 ± 2.1^a	519 ± 1.3^a	515 ± 1.5^b	0.05
Days on feed, d				
Cool season	183 ± 1.7^a	172 ± 0.9^b	172 ± 1.0^b	<0.0001
Warm season	168 ± 1.3^c	172 ± 0.8^b	177 ± 0.9^a	<0.0001
Combined seasons	167 ± 1.4^c	172 ± 0.8^b	177 ± 1.0^a	<0.0001
Age at slaughter, d				
Cool season	418 ± 1.9^b	424 ± 1.0^a	413 ± 1.1^c	<0.0001
Warm season	427 ± 1.5^a	416 ± 0.9^b	417 ± 1.1^b	<0.0001
Combined seasons	433 ± 1.5^a	421 ± 0.9^b	411 ± 1.1^c	<0.0001

[a, b, c] Means within rows without common superscripts differ ($P < 0.05$)
[1] Dry = Mean PDSI ≤ -2.00, Normal = Mean PDSI > -2.00 and < 2.00, Wet = Mean PDSI ≥ 2.00.
[2] Cool season = April and May, Warm season = June through August, Combined seasons=April through August.
[3] Least square mean ± standard error.

Table 4. Effects of drought class[1] for each growing season period[2] on carcass composition

	PDSI Drought Classes[3]			
Item	Dry	Normal	Wet	*P*-value
HCW, kg				
Cool season	320 ± 1.6[ab]	322 ± 0.8[a]	316 ± 0.9[b]	<0.01
Warm season	318 ± 1.3	318 ± 0.8	321 ± 0.9	0.12
Combined seasons	322 ± 1.3[a]	320 ± 0.8[b]	318 ± 0.9[b]	0.03
Dressing percent, %				
Cool season	61.4 ± 0.10	61.5 ± 0.05	61.6 ± 0.05	0.61
Warm season	61.6 ± 0.08	61.5 ± 0.05	61.5 ± 0.05	0.42
Combined seasons	61.6 ± 0.08	61.5 ± 0.05	61.5 ± 0.06	0.93
Backfat, cm				
Cool season	1.16 ± 0.019[ab]	1.13 ± 0.009[b]	1.21 ± 0.004[a]	<0.0001
Warm season	1.11 ± 0.015[b]	1.18 ± 0.008[a]	1.19 ± 0.004[a]	<0.0001
Combined seasons	1.11 ± 0.015[b]	1.18 ± 0.009[a]	1.19 ± 0.10[a]	<0.0001
LM area, cm^2				
Cool season	77.9 ± 0.39[b]	79.0 ± 0.19[a]	78.1 ± 0.19[b]	<0.001
Warm season	79.2 ± 0.31[a]	78.3 ± 0.18[b]	78.4 ± 0.22[ab]	0.01
Combined seasons	79.4 ± 0.31[a]	78.5 ± 0.19[b]	78.2 ± 0.22[b]	0.01
Calculated yield grade				
Cool season	2.92 ± 0.031[a]	2.84 ± 0.016[b]	2.93 ± 0.018[a]	<0.0001
Warm season	2.77 ± 0.025[b]	2.90 ± 0.015[a]	2.93 ± 0.018[a]	<0.0001
Combined seasons	2.83 ± 0.025[b]	2.89 ± 0.016[a]	2.91 ± 0.018[a]	0.02
Marbling score[4]				
Cool season	1047 ± 4.0[a]	1042 ± 2.1[a]	1035 ± 2.3[b]	0.05
Warm season	1032 ± 3.2[b]	1040 ± 1.9[a]	1042 ± 2.3[a]	0.02
Combined seasons	1038 ± 3.2[ab]	1044 ± 2.0[a]	1036 ± 2.4[b]	0.01

[a, b] Means within rows without common superscripts differ ($P < 0.05$).

[1] Dry = Mean PDSI ≤ -2.00, Normal = Mean PDSI > -2.00 and < 2.00, Wet = Mean PDSI ≥ 2.00.

[2] Cool season = April and May, Warm season = June through August, Combined seasons = April through August.

[3] Least square mean ± standard error.

[4] Trace[0]=800 Slight[0]=900 Small[0]=1000 Modest[0]=1100 Moderate[0]=1200 Slightly Abundant[0]=1300 Moderately Abundant[0]=1400.

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Winter grazing or confinement feeding heifer development strategies differ in energetics as measured by 24 hour heart rate and activity.

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ABSTRACT: A heifer's ability to thrive is partially due traits including behavioral/metabolic adaption and genetic background. Type of weaning and development program implemented creates an environment replacement heifers must adapt to flourish. This study was designed to determine if heifers developed in confinement or grazing native range use different adaption and coping processes by measuring activity such as distance traveled and percent resting time in 24 hrs along with resting heart rate and average heart rate per day. Spring-born, crossbred heifers were stratified to 1 of 2 treatments at weaning (start of Period 1): (1) fence line weaning on native range (**NR**) with self-fed salt-mineral protein supplement (n=118) and after weaning received a hand fed daily energy supplement or a self fed protein supplement or (2) weaned into a dry lot **(DL)** and fed a corn silage diet formulated to gain 0.68 kg/d (n=53). Ad libitum grass hay was made available in mid-December due to snow coverage resulting in range forage inaccessibility. Heifer BW were taken every 28d from initiation of weaning. Each month (except February and June) a cohort of 7 heifers from each treatment were fitted with equine heart rate monitors (Polar Equine RS800CX) and QSTARZ CR-Q1100P GPS tracking recorder. Data were recorded for 48 hrs. On April 9, 2014 (Period 2) when the two supplement groups grazing NR, and DL heifers were combined into a common pasture. Heifers receiving DL had greater ($P < 0.01$) BW throughout the entire study. Resting heart rate was influenced ($P<0.01$) by an interaction of period and weaning/development management. The rankings of resting heart rate were reversed from period 1 to period 2. Resting heart rate was also shown to influence BW. Resting heart rate relationship with BW was negative suggesting that lower resting heart rate is related greater body weight. The analysis suggests that for every 2.2 decline in resting HR there is an additional .45 kg of BW. This study indicates that resting heart is negatively related to body weight implying that animals with lower resting heart rate may have a production advantage.

Key Words: beef heifers, heifer development, heart rate

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Introduction

Heart rate can be interpreted as an indirect assessment of energy metabolism. It is the result of oxygen use for aerobic energy utilization to support work of the organism. Breed, body weight, growth rate, temperament, speed, gait, weather variables, experience (coping behaviors), digestive products, and proportion of muscle fiber profile affect metabolic effort (heart rate).

Brosh et al. (2007) and Aharoni et al. (2009) implemented heart rate (HR) monitors and GPS recorders along with other measurements to assess caloric costs of specific activities of different breeds. They measured the same individuals in a repeated measures approach for a yearlong appraisal. They also inferred from their results that "foraging activity increases energy requirements of animals in paddocks compared with confined animals However, there is a scarcity of data utilizing simultaneous HR and GPS recordings to assess the impacts of management schemes on consequent adaptability or fitness of animals in differing environments.

Although Brosh et. al. (2007) and Aharoni et. al. (2009) found breed influences on heart rate there are a number of other management practices unevaluated. The potential connection between management practices and carry over effects of those practices on fitness (resting heart rate) and energy expenditure (total or average heart rate) is unexplored. In spring, calving herds fall weaning of replacement heifers into a feedlot is commonly practiced due to the perceived quality of the environment created by supplying high quality feed, water, and a level of protection from climatic elements. This is in contrast to the loss of management control when heifers select their diet, cope with cold weather extremes, and choose protective topography while grazing late season native range. This study was designed to determine if heifers developed in confinement or grazing native range use different adaption and coping processes by measuring activity such as distance traveled and percent resting time in 24 hrs along with resting heart rate and average heart rate per day.

Materials and Methods

This study was conducted at the USDA-ARS Fort Keogh Livestock and Range Research Laboratory near Miles City, MT from November 2013 through August 2014. Heifers were born in the spring of 2013 in a herd managed at the site for the past 30 years. The genetic influence of

this herd is a composite (50% Red Angus, 25% Tarentaise and 25% Charolais) originating at Fort Keogh. Prior to weaning, heifers grazed alongside their dams in a 350 cowherd, utilizing native vegetation on the 22,500-ha research station. The research site is a grama-needlegrass-wheatgrass (Bouteloua-Stipa-Agropyron) mix. The long-term average precipitation is 343 mm with 85 % occurring during April through September growing season. Pastures were grazed so that forage was never limiting, water and mineral were always available (Roberts et.al 2009).

Heifers were weaned in the fall and assigned by body weight to one of two weaning treatments and after weaning to three development treatments. The treatments were (1) fence line weaning while grazing native range (**NR**) with a salt-mineral protein (n=118) and after weaning receive a hand fed daily energy supplement or a self fed protein supplement or (2) weaned into a dry lot **(DL)** and fed total mixed ration based on corn silage formulated to gain 0.68 kg/d (n=60). Heifers assigned to DL were separated from their dams and immediately transported to pens at Fort Keogh feedlot. Dry lot heifers were stratified into groups of 6 based on weaning weight. Groups were randomly assigned to 1 of 10 pens. Pens were 5.8 × 11 m in size and each pen contained 6 individual feed bunks equipped with electronic Calan gates (American Calan, Northwood, NH) to allow individual feeding. Pens were bedded with straw to provide insulation from the concrete pen floor. Heifers were allowed a minimum of 1 mo for adaptation to experimental pens and to become trained to the electronic Calan gates. (Roberts et.al 2009). The NR heifers were fence line weaned when their dams were removed from a common pasture into an adjacent pasture. A self-fed salt-mineral protein (Mulliniks et al 2012) supplement was initiated with NR prior to weaning. In December NR, heifers were allotted to two supplementation strategies. The first strategy was a continuation of the self-fed supplement they received prior to weaning (**Protein**) while the second was hand fed at 1.8 kg $hd^{-1}d^{-1}$ of a 20% CP starch and fiber supplement (**Cake**). In mid-December, snow buildup resulted in range forage inaccessibility so ad libitum grass hay was made available to NR heifers. Heifer BW was taken every 28 d from initiation of weaning to end of study. After April 9, 2014 when the two supplement groups grazing NR, and DL heifers were combined into a common pasture composed of mostly dormant forage and had access to the self-fed protein supplement until the end of May (start of Period 2).

On 9 separate weigh dates from November 2013 to August 2014, a cohort of 7 heifers from each treatment were fitted with equine heart rate monitors (HR) (Polar Equine RS800CX) and QSTARZ CR-Q1100P GPS tracking recorder. Data were recorded until batteries were exhausted (less than 48 hr). Heart rate monitors were used to determine average and resting heart rate and GPS tracking recorder was used to determine distance traveled and percentage of time loafing. A belt was custom made by Strapworks, LLC© (Eugene, Oregon) to secure the heart rate monitor and GPS to the heifer. Heifer hair was clipped along the spring of the rib between heart girth and shoulder. A water based ultrasound gel was placed on the shaved area to enhance electrical conductivity to the electrodes attached to the elastic belt. At the top of the belt in the area adjacent to the withers the HR and GPS recorders were fastened. Data were recorded every minute by heart rate (BPM) and GPS tracking recorders.

Resting heart rate was determined on heifers grazing native range by determining the mean heart rate during a span of time when GPS recorded 0 distance (heifer not moving). Heifers in the confinement pens were seldom inactive for more than a few minutes so resting heart rate was determined by averaging a minimum of 5-10 minute time span of lowest ranking heart rate readings.

Distance traveled was the accumulation of distance between each one-minute position reading. Time spent resting was sum of consecutive minutes when the GPS recorded 0 distance movement. We assumed there needed to be at least 5 accumulated minutes in a period for it to be designated as rest.

Statistical Analysis

Data were categorized into two periods, with Period 1 consisting of data collected on five dates in November 2013 through April 1, 2014 during the post weaning treatments and Period 2 consisting of data collected on four dates after all animals were combined on April 9 and managed similarly for the remainder of the sampling . Due to equipment failure, heart rate monitor data collected over the 9-weigh dates were not available for all 63 possible observations for each treatment. Minimum numbers of observations within a treatment were 19 of 35 possible in Period 1 and 17 of 28 possible in Period 2. Data were analyzed as a completely randomized design with heifer as the experimental unit using the MIXED procedure (SAS Inst. Inc., Cary, NC). Effects of treatment and period on Heart rate and GPS measurements were analyzed using a model that included main effects and interaction of treatment and period. To evaluate potential associations among the different heart rate and GPS measurements with growth, separate analyses were run for each measurement fit as a continuous variable in a model that also included BW at weaning as a covariate, and main effects and interaction of treatment and period. Interaction was removed from the model if F statistic was < 1.

Results and Discussion

Management Strategy

The impacts of weaning and development strategies on body weight gain and reproduction have been widely reported. Insight into the success or failure of management strategies may require measurement of adaption, coping ability, and the extent of carry over effects to understand how the production environment influences gain and reproduction. Activity can suggest searching behavior, stress, anticipation, sickness, and/or contentment; whereas resting heart rate is indicative of metabolic work influenced by factors such a growth rate, fitness or physical conditioning, stress, responses to climatic variables and others. After the completion of Period 1 the DL heifers weighed 38 or 54 kg more (P <0.05) than either Cake or Protein groups respectively (Table 1). At the completion of

Period 2, the differences in body weight were reduced to 14 kg and 38 kg for Cake and Protein treated heifers respectively. Heifers in the NR management scheme experienced compensatory gain in the summer grazing period. A primary stimulus effecting body weight in Period 1 is caloric density of the diet and a possible second factor could be differences in energy expenditure due to travel necessary to consume dietary ingredients and the ability to resist winter weather conditions. Whereas in Period 2 the DL heifers were heavier and therefore had higher maintenance energy requirements to sustain their greater weight. They also have not had to search and acquire their diet in Period 1 and may require an interval of time to develop efficient grazing tactics.

Heart Rate

In addition to experimental effects, uncontrolled variables identified in this study may have influenced resting heart rate such as physical conditioning, cold or hot ambient temperature, sickness, or apprehension (anticipating feeding). A higher resting HR suggests that in most cases more work is required for maintenance metabolism. Resting heart rate was influenced (P<0.01) by an interaction of period and weaning/development management (Table 2). The rankings of resting heart rate were reversed from period 1 to period 2. In period 1, the DL heifers showed a 10 to 14 beats per minute (BPM) higher (P < 0.01) resting HR than their NR herd mates. Potential factors influencing a lower HR in the NR heifers includes low rate of gain, physical conditioning due to activity and exposure to winter environmental conditions and declining maintenance energy requirements associated with negative or neutral body weight change. Additionally the Cake fed range raised heifers were found to have a four BPM higher (P < 0.01) resting HR than the Protein fed heifers. Even though the Cake and Protein heifers both grazed native range, the Cake heifers were hand fed 1.8 kg of cake at 1000 hr each day. The Cake heifers would spend most of the morning hours anticipating feeding by walking in circular routes in the area where they expect to be fed. It was not until later in the day did the Cake group focus on harvesting range vegetation. In period 2 when all hand feeding was discontinued and the DL heifers grazed with NR heifers, the DL group were found to have the lowest resting HR at 59 BPM$\pm$2.0 in contrast to the Cake and Protein heifers with a resting HR of 63$\pm$2.0. It is possible that the greater heart rate of the NR groups reflects the higher rate of gain and subsequent metabolic work required for growth. It is also interesting to speculate that the DL group adapted from a confinement situation associated with a higher resting HR to a grazing situation in a few months as demonstrated by a lower resting HR indicative of the NR management scheme.

Average heart rate, which reveals total energy expenditure, was also affected by an interaction of management strategy and period where DL treated heifers in Period 1 averaged 101+4.90, which was 22 BPM greater than the NR treated heifers. However, in Period 2 all groups had similar average heart rates.

Activity

Measurements of activity provide partial insight into the HR interactions. In Period 1 DL, heifers used less than 10% of the day resting which was less than the 30$\pm$1.5% resting time exhibited by the range-raised heifers (Table 2). The reduced time spent resting could be the product of expectations for the next delivery by the feed truck. A second influence could include slow adjustment to confinement from the extensive landscape common to the grazing environment as calves, and maybe further compounded by the pen flooring made up of concrete apron, frozen ground and bedding. In period two, the DL heifers were more similar to NR group resting 32$\pm$1.7% of the day. Distance traveled per day showed a pattern similar to time spent resting as influenced by management strategies and periods where DL heifers walked the least (P <.01) in Period 1 and were found to walk the same distance as Protein heifers in Period 2. In contrast to the Cake group that traveled the least (P <0.01). The tendency to travel further may be the product of more time spent searching and less efficient grazing.

Covariance

As expected the covariate of weaning weight was positively associated (P <0.01) with body weight (Table 3). Resting heart rate was also shown to influence BW. However resting heart rate relationship with BW was negative. The analysis suggests that for every 2.2 decline in resting HR there is an additional .45 kg of BW. As a covariate, the influence of resting heart is independent of period or management strategy. Based on this coefficient for the effect of resting HR on BW, comparing higher resting HR for the DL heifers during Period 1, to the lower rates observed in all groups when grazing in period 1 or 2, indicates an advantage for the grazing animal than the group in confinement. The advantages conferred in the grazing environment could be greater contentment supported by an extensive environment and less competition possibly associated with no delivered feed. In period 1 both DL and Cake had greater resting HR than Protein. The Protein group supplement was consumed when they choose to eat it and was not in contest after the feed truck delivery. Lastly when grazing range land the heifers are physically active, navigating undulating terrain, exploring and walking from the last grazing location to water. These activities create mild but significant exertion which the body adapts and in the process becomes more efficient and requires fewer calories over time for the same level of exertion. The combination of these influences most likely leads to a reduced resting HR providing more energy available for body weight gain.

Implications

In spite of a common perception by those who manage heifer development programs that a confined environment where feed and water are delivered is favored, analysis of resting HR and average HR results from this study suggests that confined heifers expend more energy than heifer grazing native range for maintenance metabolism and activity. We can also speculate that

multiple factors inspiring adaption in weaned range heifers combine to reduce maintenance energy requirements as demonstrated by a lower resting HR compared to a peer group of confined heifers. Activity as one of the factors influencing HR, which is also affected by management strategy and changes with the strategy. This study indicates that resting heart is negatively related to body weight implying that animals with lower resting heart rate may have a production advantage.

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Table 1. Influences of development management strategies (Treatment) in winter or summer (Period) on heifer body weight

		Treatment[1]				
Measure	Period[2]	Dry lot	Cake	Protein	SEM	*P*-value[3]
BW, kg	1	278.2	239.9	224.1	7.8	0.38
	2	312.9	298.6	274.6	7.8	

[1] Dry lot heifers in period 1 fed a total mixed ration formulated to gain 0.68 kg/d. In period 2 dry lot heifers grazed native range. The heifers in the cake group were fed at 1.8 kg $hd^{-1}d^{-1}$ of a 20% CP starch and fiber supplement daily. The protein treatment to a self fed protein (55% CP) supplement.
[2]Period 1= November 2013 to April 1, 2014. Period 2= April 9, 2014 to August, 2014.
[3]Treatment × Period

Table 2. Influences of development management strategies (Treatment) in winter or summer (Period) on heifer resting heart rate, average heart rate, % rest time and distance traveled in a 24 hr period.

		Treatment[1]				
Measurement	Period[2]	Dry lot	Cake	Protein	SEM	*P*-value[3]
Resting Heart Rate, beats/min	1	67^{a}	57^{cd}	53^{d}	2.6	0.001
	2	59^{bc}	63^{ab}	63^{ab}	2.9	
Average Heart Rate, beats/min	1	101^{a}	79^{b}	79^{b}	4.9	0.001
	2	94^{a}	96^{a}	94^{a}	5.1	
Rest Time, % of 24 h	1	8^{b}	30^{a}	30^{a}	1.5	0.001
	2	32^{a}	29^{a}	28^{a}	1.7	
Distance, km	1	2.3^{c}	3.9^{b}	3.5^{b}	0.8	0.02
	2	8.6^{a}	7.2^{a}	8.1^{a}	0.8	

[1] Dry lot heifers in period 1 fed a total mixed ration formulated to gain 0.68 kg/d. In period 2 dry lot heifers grazed native range. The heifers in the cake group were fed at 1.8 kg $hd^{-1}d^{-1}$ of a 20% CP starch and fiber supplement daily. The protein treatment to a self fed protein (55% CP) supplement.
[2]Period 1= November 2013 to April 1, 2014. Period 2= April 9, 2014 to August, 2014.
[3]Treatment × Period
[abcd] Means in rows and columns with different superscripts differ ($P < 0.05$).

Table 3. Estimates for Fixed Effects for factors influencing BW

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	223.49	27.90	120	8.01	<.0001
BW at weaning (covariate)	0.24	0.04	120	6.39	<.0001
Rest Heart Rate (covariate)	-2.23	0.68	120	-3.26	0.01
Trt = Feedlot	45.25	7.62	120	5.94	<.0001
Trt = Range + Cake	19.24	6.99	120	2.75	0.01
Trt =Range + Protein	0.00	.	.	.	.
Period 1	-47.83	6.01	120	-7.96	<.0001
Period 2	0	.	.	.	.

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Effects of dietary phytoestrogens on testicular growth and semen quality characteristics in developing Angus bulls

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ABSTRACT: The aim of this study investigated the impact of scrotal growth and semen quality parameters of bulls consuming dietary phytoestrogens versus bulls that are not exposed to phytoestrogen containing diet ingredients. Angus bulls born in consecutive years were used in 2 independent trials. Bulls born in the spring of 2014 (n = 39) and 2015 (n = 24) were stratified by weaning weight, age of dam (AOD), and sire into a soybean meal diet group (SBMTRT) or a cottonseed meal diet group (CSMCON). At weaning (d -42) bulls were assigned to treatment and adapted to concentrate diets. At d 0, 21, 54, and 86 scrotal circumference measures were collected with semen collection and assessment also being conducted at d 86. Differences in scrotal circumference were detected due to the diet × day(year) interaction. On d 54 and 86 SBMTRT in 2014 exhibited larger scrotal measures than SBMTRT in 2015 ($P \leq 0.05$). At d 54 SBMTRT scrotal measures were also greater than the CSMCON scrotal measures in 2015 ($P = 0.05$). Scrotal growth from d 0 to 21 was greater for SBMTRT across both years ($P < 0.0001$). This pattern of larger teste growth was also observed from d 54 to d 86 ($P = 0.05$), and from d 0 through d 86 ($P < 0.0001$). Variation in semen concentration was due to the diet × AOD(Year) interaction. The SBMTRT, produced from 2 - year old females, expelled higher concentrated semen samples in 2014 ($P = 0.026$) and 2015 ($P = 0.0006$), and this was inconsistent with the 2015 CSMCON out of 5+ year old cows who were higher for semen concentration ($P < 0.0001$). The diet × AOD(Year) interaction was also a source of variation for motility. The 2014 bulls from 2 and 5+ year old cows ranked higher than the CSMCON cohorts of like aged cows ($P < .0001$). This trend was not evident in 2015 however, as the CSMCON mean was greater than the SBMTRT from 3 year old cows only ($P = 0.0129$). Variation from the effect of diet for motility was also observed as well, as SBMTRT scored higher than CSMCON ($P = 0.0308$). These data suggest that dietary phytoestrogens at 10% soybean meal diet inclusion improves scrotal growth and semen quality and this is particularly evident in bulls produced by 2-year old, first calf females.

Key words: bull development, isoflavone, phytoestrogen, semen quality, soybean meal[1]

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INTRODUCTION

Although soybean meal is a viable source for protein in livestock, it contains phytoestrogens classified as isoflavones that may impact or modify the reproductive function in males (Cederroth et al., 2009). Isoflavones are polyphenolic compounds that can exert estrogen like effects on the body and are derived principally from soybeans and clover (Cardoso and Bao 2006). There are two specific isoflavones; genistein and diadzein which are thought to exert some of the most potent estrogenic hormone activities (Lephart et at., 2004). Estrogen is important in the regulation of the male reproductive tract and has direct effects on leyding cells and efferent ductule epithelium with potential effects on germ cells. Estrogen receptors are abundant throughout the body and reproductive tract, but are even more localized in the efferent ductule epithelium where sometimes the presence of estrogen is even more pronounced than in the female reproductive tract (Hess and Carnes, 2004). Hess (2003) observed that estrogen is found in abundance in the testis, rete testis fluid, and semen of many species such as ram, bull, stallion and boar. Therefore, a disruption of the estrogen receptors can have adverse effects on sperm production and morphology.

There is currently little published data that explains the effects of these soy based isoflavones on ruminant livestock, in particular pre-pubertal bulls. The objective of the current study is to investigate the effects of phytoestrogens in dietary soybean meal on scrotal measures, and semen quality measure in growing bulls.

MATERIALS AND METHODS

All animal handling procedures and data collection methods were approved by the Angelo State University Institutional Animal Care and Use Committee (AUP # 14-05). Data used in this study was collected from spring born, pre-pubertal Angus bulls (n = 63) which represents 2 consecutive, but independent, years of data collection (2014: n = 39; 2015: n = 24). In each year, bulls were stratified by weaning weight, age of dam (AOD), and sire across 1 of 2 project diet groups: a phytoestrogen consuming group with 10% soybean meal inclusion as a diet protein source (SBMTRT), and a phytoestrogen naïve group with 10% cottonseed meal inclusion as a diet protein

source (CSMCON). The same study rations were used in both years with weekly batch samples being composited across both years for nutrient composition analysis. Study diets were formulated, and confirmed to be isocaloric and isonitrogenous with all bulls having *ad libitum* access to fresh water supply throughout the duration of the observation days during each year. Trial ration information is presented in Table 1.

Table 1. Ingredient and nutrient composition (DM basis) of CSM ration and SBM ration

	CSM Diet %	SBM Diet %
Ingredient		
Cracked Corn	30	30
Cottonseed meal, dry	10	0
Soybean meal, dry	0	10
Corn Glutten Feed, pellet	15	15
Cottonseed Hulls, dry	18	22.5
Alfalfa Pellet	20.5	16
Molasses	4	4
Mineral Premix[1]	2.5	2.5
Nutrient Composition		
DM	88.61	88.85
Crude Protein	17.62	17.92
Crude Fat	3.82	3.78
ADF	24.2	24.19
NDF	35.64	35.26
Calcium	0.96	0.92
Phosphorus	0.47	0.43

[1]Premix: 17.5 – 19% Ca, 18.1 – 20.6% NaCl, 1075 ppm Mn, 1780 ppm Zn, 3.95 ppm Se, 89,187.09 IU/kg Vitamin A, 29,728.03 ppm Vitamin D, 493.83 ppm Vitamin E.

At d -42 in each year, all bulls were weaned, weighed, then sorted and comingled into their respective diet groups. All bulls were initially fed the cottonseed meal diet for backgrounding and concentrate diet adaptation purposes. At d 0, basal scrotal measurements were collected and the SBMTRT was gradually transitioned to the SBMTRT treatment diet. Scrotal circumference measures were observed again at d 21 and 54. On d 79 an initial semen sample was collected via electro-ejaculator and discarded to verify a reproduction tract dispel of all bulls. At d 86, a final scrotal circumference was documented, semen samples were collected via electro-ejaculator, and semen quality characteristics were recorded. Semen motility parameters were subjectively assessed utilizing the same trained personnel in both observation years using a 5-point scale as described in Table 2. Semen concentration measures were counted via Dupree model 591B densimeter and recorded as number of spermatozoa per milliliter at the time of ejaculation. Trial procedures and day of the trial across the respective year is presented in Table 3.

Table 3. Procedure days and associated dates across years

	2014 – 2015		2015 – 2016	
Procedure	**Day**	**Date**	**Day**	**Date**
Wean, weigh, sort, and concentrate diet adaptation	-42	10/01/2014	-42	09/30/2015
Scrotal Measure, treatment diet begins	0	11/12/2014	0	11/11/2015
Scrotal Measure	21	12/03/2014	21	12/02/2015
Scrotal Measure	54	01/05/2015	54	01/04/2016
Initial semen collection for tract dispelling	79	1/30/2015	79	01/29/2016
Scrotal Measure, Semen evaluation	86	02/06/2015	86	02/05/2016

Statistical Analysis Data from both years was compiled and mixed model procedures of SAS (v. 9.2; SAS inst, Inc., Cary, NC) were used to analyze scrotal circumferences with a model that includes the fixed effects of day(nested within year), year (2014 or 2015), diet (SBMTRT or CSMCON), age of dam (2, 3, 4, 5+), sire, d -42 weight as a covariate, and two-factor interactions. These models were analyzed as repeated measures with a first order autoregressive covariance structure. Changes in scrotal circumference (SC) were calculated by time period (Period 1 scrotal growth = difference of d 0 SC and d 21 SC: Period 2 scrotal growth = difference of d 21 SC and d 54 SC: Period 3 scrotal growth = difference of d 54 SC and d 86 SC; POSTWEAN SC Growth = difference of d 0 SC and d 86 SC). Scrotal growth within time periods, semen concentration (transformed to a log base of 10 for analysis), and semen motility scores were analyzed using a similar model but excluding the repeated measures statements. In all models, main effects with ($P \geq 0.15$) and interacting terms with ($P \geq 0.25$) were removed from final analysis in all models. Least squares means were separated using / PDIFF option and P – values ≤ 0.05 were considered different.

Results and Discussion

Scrotal Circumference Differences in least squares means (LSMEANS) for scrotal circumference (SC) measures were observed at d 54 and d 86 for the Diet × Day(Year) interaction. A similar increasing pattern for all diet by year combinations across days was observed and it is of interest that at day 54, the SBMTRT in 2014 recorded higher SC means as compared to SBMTRT and CSMCON in 2015 ($P = 0.05$ and $P = 0.03$ respectively). At day 86 however, differences in SC were only detected between the SBMTRT in 2014, which was higher than SBMTRT in 2015 ($P = 0.05$). These results are presented in Figure 1.

Figure 1. Scrotal Measures, in cm for Diet × Day(Year)

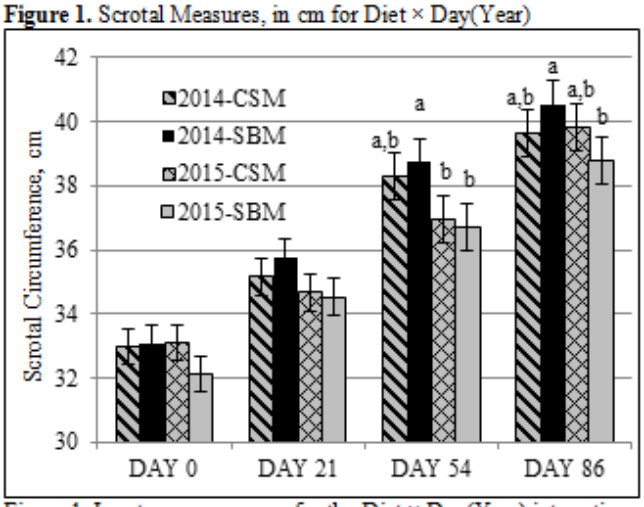

Figure 1. Least squares means for the Diet × Day(Year) interaction on scrotal measures in cm.
[a, b] superscripts designate differences ($P \leq 0.05$) of diet by year within day.

While no differences in SC were observed between the diet groups in the same year and on like days, the rate of testicular growth of SBMTRT in period 1, period 3, and in the overall postweaning period indicates enhanced teste development as compared to CSMCON contemporaries ($P < 0.0001$, $P = 0.01$, $P < 0.0001$ and respectively) (Figure 2.).

Figure 2. Scrotal increases per observation period in cm: Main effect of Diet

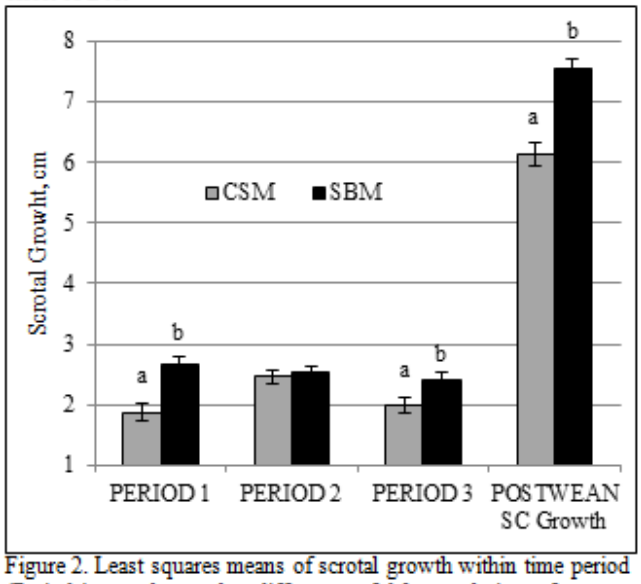

Figure 2. Least squares means of scrotal growth within time period (Period 1 scrotal growth = difference of d 0 scrotal circumference and d 21 scrotal circumference: Period 2 scrotal growth = difference of d 21 scrotal circumference and d 54 scrotal circumference: Period 3 scrotal growth = difference of d 54 scrotal circumference and d 86 scrotal circumference; POSTWEAN SC Growth = difference of d 0 scrotal circumference and d 86 scrotal circumference).
[a, b] superscripts designate differences ($P \leq 0.05$) within time period.

These data would reflect similar results presented by Yuan et al. (2012), in which boar pigs consuming 500ppm isoflavone supplement were 40% lower in scrotal size index calculations as compared to a negative control group, and 58% lower in scrotal size index calculations as compared to boars consuming 250ppm isoflavone supplement. Therefore implying that the level of isoflavone exposure in these data is not high enough to cause deleterious effects to SC but dietary soybean meal at a 10% ration inclusion rate augments teste SC accretion.

Semen Sample Sperm Cell Concentration It is well understood that age of dam (AOD) is an important point to consider when discussing bull development procedures and performance adjustment factors (Koch and Clark, 1995; Lunstra et al,. 1998). Differences and the LSMEANS of the transformed (log10) semen concentration variable due to the diet × AOD (nested within year) interaction are presented in Table 4. In our study, differences were observed in bulls produced by 2-year old dams where bulls in the SBMTRT produced semen samples more heavily concentrated than the CSMCON born to 2 year old dams in 2014 and 2015 ($P = 0.026$ and $P = 0.0006$ respectively). But in 2015, CSMCON from the 5+ AOD designation expelled semen ejaculate that was 7.47% more concentrated than SBMTRT born to 5+ year old females ($P < 0.0001$). Sperm cell concentration in ejaculate is an important criteria of semen charactersitcs to qualify fertile males for breeding purposes (Graffer et al., 1988). This data suggests that moderate phytoestrogen exposure in bulls born to first calf females offers decisive advantages in sperm cell proliferation and semen concentration.

Sperm Motility the main effect of diet in the semen motility scoring analysis is summarized in figure 3.

Figure 3. Semen motility scores, Main effect of Diet

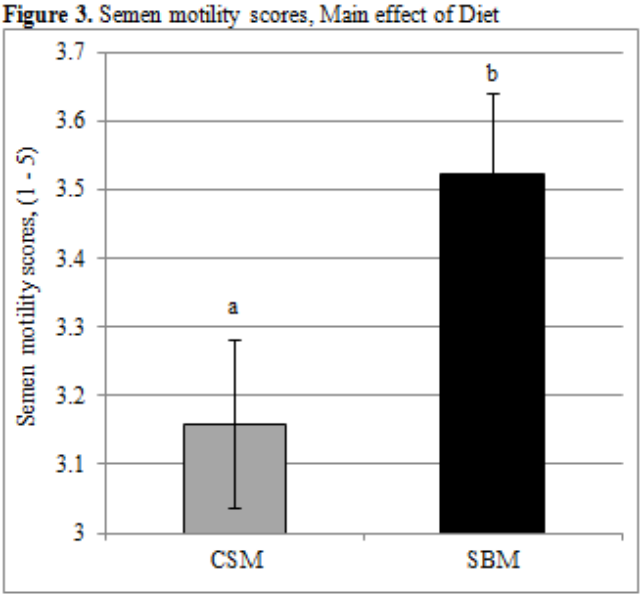

Figure 3. Least squares means of Semen motility scores (1 – 5) with scores of 1 being least motile and scores of 5 being most motile.
[a, b] superscripts designate differences ($P \leq 0.05$).

It was observed that the LSMEANS for motility of SBMTRT was 3.52, which is higher than CSMCON motility scores of 3.16, and this was a significant source of variation ($P = 0.0308$) in the analysis. Additionally, the Diet × AOD(Year) interaction was also a source of model

variation and LSMEANS are presented in Table 5. In the 2014 data, the SBMTRT produced by first parturition, 2 - year old females and cows that are ≥ 5 years of age recorded higher semen motility scores than CSMCON ($P < 0.0001$). But in the 2015 data, the only observable differences were in the bulls from 3 - year old cows, as the CSMCON scored higher than the SBMTRT ($P = 0.0129$) and specific reasons for these conflicting results is not clearly interpreted. It should be noted that Alexander (2008) concludes that bulls should have a minimum of 30% progressive motility estimation to be deemed as a satisfactory breeder. In these data, semen motility scores of 2 or higher represent a 30% motility equivalent and therefore, all bulls in these study groups were deemed as acceptable potential breeders.

IMPLICATIONS

These results support the inclination of positive aspects of exposing growing beef bulls to dietary phytoestrogen compounds by providing soybean meal as a protein source at the 10% inclusion rate of a diet of growing bulls. Additional research objectives should investigate variable rates of phytoestrogen intake, alternative timing of postnatal phytoestrogen exposure, and the potential effects of prenatal phytoestrogen exposure by modifying maternal consumption of phytoestrogens.

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Table 2 Semen grading scores for semen motility parameters[a]

Scale	Grade	Characteristics
5	(+++++) Excellent	More than 80% of the sperm show vigorous motion. Swirls are formed due to the movements of the sperm. The movements are rapid and changing and hard to observe individual sperm samples in undiluted semen.
4	(++++) Very good	About 70-80% of the sperm show vigorous motion which causes waves and eddies but not as vigorous as the excellent grade.
3	(+++)	About 45-70% of the sperm are in motion. Motion is vigorous. Waves and eddies are formed slow across the sample.
2	(++) Fair	30-40% of the spermatozoa are in motion. Movements are vigorous. No waves or eddies present.
1	(+) poor	Little to no mobility found. < 20% of the spermatozoa are in motion. Not progressive and little oscillation.

Adapted from Hossain et al. (2012). This table illustrates the measure of motility of semen samples presented in this report.

Table 4. Semen Concentration (Transformed to log base of 10) of Diet × AOD(Year)

	2014				2015			
AOD	CSM	SBM	SEM	*P*=	CSM	SBM	SEM	*P*=
2	^{a}8.0974	^{b}8.4654	0.1649	0.0266	^{x}7.5185	^{y}8.6702	0.3297	0.0006
3	8.2122	8.0944	0.2692	0.6621	8.0211	8.0302	0.1904	0.9617
4	8.5436	8.5026	0.1461	0.7792	8.7372	8.2945	0.3297	0.1808
5+	8.455	8.532	0.1297	0.5535	^{x}8.7756	^{y}8.1655	0.1412	<.0001

Table 4. Least squares means of semen concentration (Transformed to log base of 10) of the Diet × AOD(Year).
$^{a, b}$ superscripts designate differences ($P \leq 0.05$) of 2014 diet effects within respective AOD.
$^{x, y}$ superscripts designate differences ($P \leq 0.05$) of 2015 diet effects within respective AOD.

Table 5. Semen Motility Scores of Diet × AOD(Year)

	2014				2015			
AOD	CSM	SBM	SEM	*P*=	CSM	SBM	SEM	*P*=
2	^{a}2	^{b}3.75	0.3453	<.0001	4	5	0.6907	0.1491
3	2	3	0.564	0.0776	^{x}3.3333	^{y}2.3333	0.3988	0.0129
4	3.1429	3	0.3061	0.6412	5	4	0.6907	0.1491
5+	^{a}2.2857	^{b}3.5	0.2717	<.0001	3.5	3.6	0.2957	0.7356

Table 5. Least squares means of semen motility scores for the Diet × AOD(Year) effect.
$^{a, b}$ superscripts designate differences ($P \leq 0.05$) of 2014 diet effects within respective AOD.
$^{x, y}$ superscripts designate differences ($P \leq 0.05$) of 2015 diet effects within respective AOD.

RUMINANT NUTRITION

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Effects of capsaicin source on blood capsaicin, glucose and insulin concentrations, rumen fermentation and nitrogen balance of sheep[1]

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ABSTRACT: This study evaluated the bioavailability of ruminally-protected capsaicin, and potential effects on rumen microbial fermentation, diet digestibility, and N retention of sheep. Twenty-one wether lambs (36.1 ± 1.0 kg BW) were used in 2 experimental periods (19 d each) based on BW (9 and 12 lambs in period 1 and 2, respectively). From d 1 to 7 of each period, lambs were adapted to indoor individual pens, and then moved to metabolism crates from d 8 to 19. Lambs were fed twice daily alfalfa hay and 1 of 3 supplements containing no capsaicin (**CON**), unprotected capsaicin (**DCAP**), or ruminally-protected capsaicin (**RPCAP**). On d 14 and 19, blood samples were collected at 0, 0.5, 1, 2, 4, 8, and 12 h, and on d 19 rumen fluid samples were collected 4 h after supplement delivery. Feces and urine were collected once daily for 5 d. The experiment was a randomized complete block, and the model included treatment, h, and treatment × h. Capsaicin was not detected in serum of lambs, and no treatment × h interactions ($P \geq 0.06$) occurred for plasma glucose or insulin on d 14 or 19. Total VFA concentrations were lowest for DCAP, intermediate for RPCAP, and greatest for CON ($P = 0.05$). Acetate proportions (mol/100 mol) tended to be lower ($P = 0.06$) for DCAP than CON and RPCAP. Fecal excretions of DM, OM, NDF, and ADF were lower ($P < 0.01$), and DM, OM, NDF, and ADF digestibility (% of intake) were greater ($P < 0.01$) for lambs fed DCAP than CON and RPCAP. Nitrogen intake, fecal and urinary N, N digestibility, and N retention were not affected ($P \geq 0.15$) by treatments. Greater differences in rumen VFA concentrations between DCAP and CON than RPCAP and CON suggest that shifts in microbial fermentation were greater when capsaicin was not ruminally-protected in a prill. Undetectable capsaicin in serum of lambs receiving capsaicin, and minimal effects of RPCAP on N balance indicates the concentration of capsaicin provided may not have entered systemic circulation. This could be due to potential microbial breakdown, inability to be absorbed, or rapid post-absorptive metabolism. Further work is warranted to develop a source of absorbable capsaicin that will withstand degradation in the rumen to allow an increase in blood capsaicin to serve as a potential anti-inflammatory supplement in ruminants.

Key words: capsaicin, nitrogen retention, ruminally-protected, sheep

INTRODUCTION

Capsaicin, the pungent compound in chili peppers, elicits anti-inflammatory effects in mammals. Dogan et al. (2004) reported that an intraperitoneal dose (5 mg/kg BW) of capsaicin modulates fever in rats subjected to lipopolysaccharide (LPS). Capsaicin does so by decreasing production of pro-inflammatory cytokines via a peroxisome proliferator activated cascade. Demirbilek et al. (2004) reported lower circulating IL-6 and tumor necrosis factor-α levels in septic rats receiving s.c. capsaicin (1 mg/kg BW) vs. no capsaicin. Therefore, capsaicin could potentially decrease inflammation associated with stress and disease in ruminant livestock.

Samuelson et al. (2014) evaluated capsaicin supplementation (0.74 mg/kg BW) in LPS-challenged steers, and concluded that capsaicin-supplemented steers did not respond immunologically different from un-supplemented steers exposed to LPS. Lack of response to capsaicin in this study is in contrast to anti-inflammatory effects observed in previous studies (Demirbilek et al., 2004; Dogan et al., 2004). We believe that the administration method by Samuelson et al. (2014) may explain the lack of response to capsaicin. Samuelson et al. (2014) administered capsaicin via dietary supplementation of jalapeño powder which may have been subjected to microbial degradation resulting in a lower post-absorptive supply. This hypothesis is supported by Alford et al. (2014) who showed that the same jalapeño powder altered VFA profiles and increased gas production by rumen microorganisms in an in vitro system. Furthermore, capsaicin could not be detected after incubation with rumen fluid. The objectives of this study were to evaluate the bioavailability of ruminally-protected capsaicin, and to evaluate potential effects on rumen microbial fermentation, diet digestibility, N retention, and blood concentrations of capsaicin, insulin, and glucose of sheep.

MATERIALS AND METHODS

Animals, Design, and Treatments

The New Mexico State University Institutional Animal Care and Use Committee approved all procedures for this experiment. Twenty-one wether lambs (36.1 ± 1.0 kg initial BW) were divided into 2 blocks based on BW (9 lambs in block 1 and 12 lambs in block 2). The experimental period for each block was 19 d. From d 1 to 7, lambs were housed

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in indoor individual pens for adaptation to feeding and supplementation, and then in metabolism crates from d 8 to 19. Lambs had ad libitum access to water and were fed twice daily alfalfa hay (44% NDF and 14.4% CP for block 1; 49% NDF and 13.2% CP for block 2) and 1 of 3 dietary treatments. Treatments (Table 1) were corn-based supplements containing no capsaicin (**CON**), an unprotected dietary capsaicin source (**DCAP**), or a ruminally-protected capsaicin source (**RPCAP**). The DCAP contained jalapeño powder (capsaicin = 850 mg/kg), and the RPCAP contained a novel ruminally-protected capsaicin prill. The CON and DCAP included a 'control' prill similar to the ruminally-protected capsaicin prill, but contained no capsaicin. Supplements containing capsaicin (DCAP and RPCAP) were fed in amounts to supply capsaicin at 1.0 mg/kg BW. Because of the pungent effects of capsaicin, lambs were adapted to 50% of their allotted dietary treatment amounts on d 8 to 10, and were fed 100% of their allotted treatment from d 11 to 19. The daily amount of total diet DM (65% alfalfa hay and 35% supplement) offered was 1.5% of BW.

Sample Collections

On d 14 and 19 of each block, blood samples were collected from the jugular vein of lambs at 0, 0.5, 1, 2, 4, 8, and 12 h after feeding of dietary treatments. Blood was collected into serum separator tubes (16 × 100 mm; BD Vacutainer SST, Franklin Lakes, NJ) and allowed to coagulate at room temperature for 30 min. Additionally, blood was collected into heparinized plasma tubes (16 × 100 mm; Monoject, Tyco Healthcare Group LP, Mansfield, MA) and immediately placed on ice. All tubes were then centrifuged (Eppendorf Centrifuge 5804) for 20 min at 2,000 × g. Serum and plasma were aliquoted into polypropylene vials and stored at -20°C for later analysis.

Representative samples of hay were collected daily, and supplement samples for each treatment were collected at mixing and stored at -20°C. There were no feed refusals throughout the sample collection period. Total fecal and urinary were collected once daily for 5 d (from d 14 to 19). Urine was collected via a funnel into 20-L plastic buckets containing 50 mL of HCl (6 *N*) to reduce NH_3 loss. Feces were collected into metal pans attached to metabolism crates. Total weights of the daily output of urine and feces were recorded, and 10% of daily urine and 100% of daily feces were frozen at -20°C. At the end of the experimental period, samples of feed refusals, feces, and urine were thawed and composited for each lamb. On d 19, samples of rumen contents were collected via oral lavage 4 h after dietary treatment supplementation. The pH of the rumen fluid was immediately recorded using a portable pH meter (Mettler-Toledo AG, Schwerzenbach, Switzerland), and 2 aliquots (10-mL each) of rumen fluid were collected and immediately frozen at -20°C.

Sample Analysis

Serum samples were plated in 96-well plates (Evergreen Scientific, Los Angeles, CA) and analyzed for glucose via a commercially available hexokinase reagent (Infinity TR15241, Thermo Scientific, Pittsburgh, PA), glucose standard (Infinity TR, Thermo Electron, Pittsburgh PA), and control serum (Infinity TR4001, Thermo Electron, Pittsburgh, PA). Samples were read on a plate reader (Elx808, BioTEK Instruments Inc., Winooski, VT) at 37°C at a wavelength of 340 nm. Serum was analyzed for insulin via solid-phase RIA (Camacho et al., 2012) with commercially available antibody coated tube technology (MP Biomedicals, Santa Ana, CA).

Determination of capsaicinoid concentrations in serum was performed using reverse phase high performance liquid chromatography (RP-HPLC). To separate capsaicinoids from most serum components, 1 mL of serum was mixed with 9 mL of 100% methanol in a glass tube, and then chilled to 0°C for 15 min. Samples were centrifuged (Beckman Coulter, Allegra 25R) at 15,000 × g for 15 min at 4°C. The supernatants were decanted into amber HPLC vials and analyzed. The HPLC (included a Waters 717 Plus Auto-Sampler and a Waters 600 Controller) was equipped with a C18 column (Waters, Nova-Pak C18, 3.9 × 150 mm). Each sample (50 µL) was analyzed using 0.1% (v/v) trifluroacetic acid solution in deionized water and acetonitrile (with 0.1% trifluroacetic acid) as the A and B solvents, respectively. Solvent A:B gradients were 90:10 and 0:100 at 0 and 15 min, respectively. The column temperature was 50°C, and flow rate was 1.2 mL/min. Excitation was at 280 nm, and fluorescence was monitored at 338 nm using a Waters 2475 fluorescence detector (gain was set to 100).

Supplements were analyzed by a commercial laboratory (SDK Laboratories, Hutchinson, KS). Fecal samples for each lamb and hay were weighed and dried for 72 h at 55°C in a forced-air oven (Blue M Electric Company, Blue Island, IL), allowed to air equilibrate overnight, and then weighed for moisture loss. Fecal, and hay samples were then ground in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) to pass a 2-mm screen. Ground samples were placed in an oven at 105°C for 24 h (Model 845, Precision Scientific Group, Chicago, IL) to determine DM, and were placed in a 550°C muffle furnace (Sybron Thermolyne, F-A1730, Thermolyne Co., Dubuque, IA) for 8 h to determine ash. Dried fecal samples and hay were analyzed for NDF, ADF, and N by a commercial laboratory (SDK Laboratories).

Frozen rumen fluid was thawed and centrifuged (Eppendorf; Newport, CA) at 20,000 × g for 10 min, and a 200 µL solution of 25% (w/v) metaphosphoric acid and 0.216% (v/v) 2-ethylbutyrate was added to 1 mL of supernatant. Gas chromatography (Agilent 7890, Agilent Technologies, Santa Clara, CA) was used for VFA analysis according to May and Galyean (1996). Similarly, 750 µL of 0.6 *N* HCl was added to 250 mL of centrifuged rumen fluid for NH_3 analysis via colorimetric assay described by Broderick and Kang (1980).

Statistical Analysis

The experiment was a randomized complete block design (lambs were divided into 2 blocks based on BW and date in metabolism crates). All data were analyzed using mixed models (SAS Inst. Inc., Cary, NC) with lamb as the

experimental unit. The statistical model for rumen parameters, digestibility and N balance included treatment, and the repeated measures model for blood parameters included treatment, h, and treatment × h interactions (covariance structure = compound symmetry). Contrasts were used to compare CON with the average for treatments containing capsaicin (DCAP and RPCAP). Treatments differences were considered significant when $P < 0.05$.

RESULTS

Capsaicinoids were not detected in serum of lambs, and no treatment × h interactions ($P \geq 0.06$) occurred for plasma concentrations of glucose or insulin on d 14 or 19 (Table 2). Rumen pH tended to be greater ($P = 0.08$), and total VFA concentrations were lower ($P = 0.05$) for DCAP and RPCAP than CON (Table 3). Molar percentages of acetate tended to be lower ($P = 0.06$) for DCAP than CON and RPCAP. Molar percentages of propionate, isobutyrate, butyrate, isovalerate, and valerate, acetate to propionate ratio, and rumen NH_3 concentrations were not different ($P \geq 0.11$) among treatments.

Dry matter and OM intake were greater ($P < 0.01$), and NDF and ADF intake were lower ($P < 0.01$) for lambs receiving DCAP than CON and RPCAP (Table 4) because of nutrient composition of supplements (Table 1). Fecal excretion of DM, OM, NDF, and ADF were lower ($P < 0.01$), and DM, OM, NDF, and ADF digestibility (% of intake) was greater ($P < 0.01$) for lambs fed DCAP than CON and RPCAP. Nitrogen intake, fecal and urinary N excretion, N digestibility, and N retention were not affected ($P \geq 0.15$) by dietary treatments.

Table 1. Dietary supplements fed to lambs

	Treatments		
Item	CON	DCAP	RPCAP
Ingredient, % DM			
Cracked corn grain	42.7	42.7	42.7
Ground Alfalfa Hay	37.4	15.0	37.4
Molasses	10.0	10.0	10.0
Mineral premix[1]	2.0	2.0	2.0
Control prill[2]	7.9	7.9	-
Jalapeño powder	-	22.4	-
Capsaicin prill[3]	-	-	7.9
Nutrient, % DM			
OM	93.0	93.0	88.5
NDF	24.1	20.5	24.5
ADF	17.1	14.9	17.7
CP	10.0	10.0	10.4
Capsaicin, mg/kg DM	0	67	75

[1]Oñate sheep mineral contained: 14 to 16.5% Ca; ≥ 11% P; 11 to 13.5% salt; ≥ 0.50% Mg; ≥ 0.10% K; ≥ 15 mg/kg Se; ≥ 1980 mg/kg Zn; ≥ 660 KIU/kg vitamin A; ≥ 165 KIU/kg vitamin D; ≥ 1.32 KIU/kg vitamin E (Oñate Feed Co., Albuquerque, NM).
[2]Similar to the ruminally-protected capsaicin prill, but contained no capsaicin.
[3]Ruminally-protected capsaicin prill.

DISCUSSION

Capsaicinoids were not detected in blood samples of lambs receiving either the DCAP or RPCAP treatments. Possible explanations are the sources of capsaicin were not protected from microbial degradation in the rumen, or the sources of capsaicin were not absorbed from the gastrointestinal tract. Additionally, post-absorptive capsaicin may have been rapidly metabolized thereby limiting detectable concentrations in the venous blood at the time points of sample collection. According to O'Neill et al. (2012), capsaicin has a half-life of approximately 47 min in systemic circulation when orally administered to rats.

Capsaicin can affect the pancreas and other peripheral organs under neuroendocrine control (Yamaguchi, 1992). Tolan and Morrison (2001) demonstrated that capsaicin had a hypoglycemic effect in dogs by increasing insulin and decreasing blood glucose levels. In the current study, no changes in glucose and insulin concentrations together with undetectable capsaicin in blood of lambs suggest that capsaicin was not absorbed from the gastrointestinal tract in concentrations that would elicit an effect on these organs. It should be noted that the amounts of capsaicin administered to dogs by Tolan and Morrison (2001) were 57-fold greater than those supplied to lambs in the current study. The capsaicin amount (1 mg/kg BW) supplied to lambs was based on the amount that elicited anti-inflammatory effects in rats (Demirbilek et al., 2004).

Undetectable capsaicin in the blood of lambs could be due to the sources of capsaicin in the dietary supplements being subjected to microbial degradation in the rumen. Rumen fluid samples of lambs supplemented with DCAP and RPCAP had lower VFA concentrations than CON lambs, which is indicative of a shift in microbial fermentation in the presence of capsaicin. Concentrations of VFA were also lower for lambs supplemented with DCAP than RPCAP, which suggests that shifts in microbial fermentation were greater when capsaicin was not ruminally-protected in a prill. This is supported by lower molar percentages of acetate in rumen fluid of lambs supplemented with DCAP, but not RPCAP, when compared to CON. Our results are consistent with the in vitro work by Cardozo et al. (2005) where they demonstrated that capsaicin decreased total VFA concentrations and acetate proportions at a rumen fluid pH of 5.5. However, Cardozo et al. (2005) also demonstrated that capsaicin increased acetate production at a rumen fluid pH of 7.0, a response also observed by Alford et al. (2014) in an in vitro batch culture system.

Shifts in rumen microbial fermentation in response to DCAP supplementation coincide with changes in total tract nutrient digestibility of lambs. Interestingly, lower total VFA and molar percentages of acetate in rumen fluid of DCAP-supplemented lambs was associated with greater total tract digestibility of DM, OM, NDF, and ADF for lambs fed DCAP compared to CON and RPCAP. Greater total tract digestibility was in part because of lower fecal excretion of DM, OM, NDF, and ADF for lambs fed DCAP compared with CON and RPCAP. In block 1 and 2, all lambs were fed (as-fed basis) on average 199 and 182 g/d of supplement, respectively. It is important to note that the

DCAP supplement was formulated to provide lambs with 1 mg/kg capsaicin, which took into account differences in DM between the alfalfa hay and jalapeño powder, nonetheless differences in DMI were observed among treatments. Therefore, differences in DMI among lambs fed CON, DCAP, and RPCAP is because of numerical differences in DM percentages among supplements. Similarly, lower NDF and ADF intakes for lambs fed DCAP is associated with lower concentrations of NDF and ADF because of the replacement of ground alfalfa hay for jalapeño powder. Nitrogen balance was not different among treatments, which could indicate that, regardless of a shift in energy metabolism (shifts in total VFA production), overall protein metabolism was not affected.

Conclusions

No evidence of capsaicin in serum of treated lambs, as well as minimal effects on N balance, indicate the supplements fed to lambs did not provide a sufficient source of absorbable capsaicin. Possible explanations are ruminal microbial degradation, insufficient intestinal absorption, or rapid post-absorptive metabolism of the sources of capsaicin. Further work is warranted to develop a source of absorbable capsaicin that will withstand degradation in the rumen and allow greater bioavailability of the molecule to serve as a potential anti-inflammatory supplement in ruminants.

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Table 2. Serum concentrations of capsaicin, glucose, and insulin in lambs supplemented with capsaicin

	Treatments[1]				*P*-value[2]			
	CON	DCAP	RPCAP	SEM	TRT	HOUR	TRT × HOUR	CON vs CAP
Serum on d 14								
Capsaicin, ng/dL	ND[3]	ND	ND	-	-	-	-	-
Glucose, mg/dL	52.4	55.1	53.7	2.57	0.75	0.09	0.31	0.53
Insulin, ng/mL	0.637	0.799	0.639	0.306	0.15	<0.01	0.28	0.31
Serum on d 19								
Capsaicin, ng/dL	ND	ND	ND	-	-	-	-	-
Glucose, mg/dL	53.1	50.7	50.1	3.38	0.63	<0.01	0.06	0.35
Insulin, ng/mL	0.578	0.643	0.621	0.101	0.68	<0.01	0.32	0.41

[1]Treatments (Table 1) were corn-based supplements containing no capsaicin (CON), a dietary capsaicin source (DCAP), or a ruminally-protected capsaicin source (RPCAP). The DCAP contained jalapeño powder (capsaicin = 850 mg/kg), and the RPCAP contained a novel ruminally-protected capsaicin prill.

[2]TRT = main effect of treatment; HOUR = main effect of h (data not shown); TRT × HOUR = treatment by h interaction; CON vs CAP = contrast for treatments containing capsaicin (DCAP and RPCAP) vs control (CON).

[3]ND = not detected.

Table 3. Rumen pH, and concentrations of NH_3, total VFA of lambs supplemented with capsaicin

	Treatments[1]				*P*-value[2]	
Item	CON	DCAP	RPCAP	SEM	TRT	CON vs CAP
pH	6.32	6.53	6.52	0.24	0.08	0.02
NH_3, m*M*	2.12	1.41	1.92	0.23	0.11	0.13
Total VFA, m*M*	109.7[a]	83.8[c]	94.8[b]	6.94	0.05	0.02
Acetate, mol/100mol	68.6	66.4	68.3	1.02	0.06	0.14
Propionate, mol/100mol	16.2	17.2	16.5	0.53	0.42	0.34
Isobutyrate, mol/100mol	1.35	1.51	1.34	0.06	0.12	0.37
Butyrate, mol/100mol	11.8	12.6	11.9	0.81	0.42	0.42
Isovalerate, mol/100mol	1.09	1.22	1.08	0.12	0.64	0.66
Valerate, mol/100mol	0.89	0.98	0.81	0.08	0.25	0.97
Acetate:Propionate	4.22	3.88	4.16	0.15	0.27	0.30

[1]Treatments (Table 1) were corn-based supplements containing no capsaicin (CON), a dietary capsaicin source (DCAP), or a ruminally-protected capsaicin source (RPCAP). The DCAP contained jalapeño powder (capsaicin = 850 mg/kg), and the RPCAP contained a novel ruminally-protected capsaicin prill.
[2]TRT = main effect of treatment; CON vs CAP = contrast for treatments containing capsaicin (DCAP and RPCAP) vs CON.
[a,b,c]Means in rows with different superscript letters differ ($P < 0.05$).

Table 4. Intake and digestibility of DM, OM, NDF and ADF, and N retention of lambs supplemented with capsaicin

	Treatments[1]				*P*-value[2]	
	CON	DCAP	RPCAP	SEM	TRT	CON vs CAP
Intake, g/d						
DM	487[c]	489[a]	487[b]	23.7	<0.01	<0.01
OM	440[b]	443[a]	440[b]	12.7	<0.01	<0.01
NDF	189[a]	183[b]	189[a]	0.52	<0.01	<0.01
ADF	145[a]	141[b]	145[a]	3.08	<0.01	<0.01
N	10.3	10.3	10.3	1.32	0.25	0.63
Feces, g/d						
DM	158[a]	142[b]	164[a]	5.13	<0.01	0.33
OM	137[a]	123[b]	141[a]	3.86	0.01	0.31
NDF	89.7[a]	78.0[b]	92.8[a]	2.82	<0.01	0.22
ADF	67.7[a]	58.0[b]	70.2[a]	5.20	<0.01	0.23
N	2.89	2.78	3.06	0.20	0.15	0.82
Digested, g/d						
DM	328[b]	347[a]	323[b]	20.4	<0.01	0.23
OM	303[b]	320[a]	299[b]	12.5	<0.01	0.20
NDF	99.1	105	96.5	2.80	0.11	0.63
ADF	76.8	82.9	74.9	2.62	0.07	0.48
N	7.40	7.48	7.25	1.14	0.35	0.92
Digestibility, % of intake						
DM	67.4[b]	70.8[a]	66.2[b]	1.10	<0.01	0.31
OM	68.8[b]	72.2[a]	67.9[b]	1.04	0.01	0.29
NDF	52.5[b]	57.4[a]	51.0[b]	1.49	0.01	0.37
ADF	53.3[b]	58.9[a]	51.7[b]	2.74	0.01	0.31
N	71.6	72.6	70.0	2.02	0.22	0.77
Urinary N, g/d	7.10	7.38	8.04	0.47	0.30	0.30
Retained N, g/d	0.250	0.068	-0.812	1.09	0.39	0.34
N retention, % of intake	1.76	-0.360	-10.2	11.2	0.31	0.32

[1]Treatments (Table 1) were corn-based supplements containing no capsaicin (CON), a dietary capsaicin source (DCAP), or a ruminally-protected capsaicin source (RPCAP). The DCAP contained jalapeño powder (capsaicin = 850 mg/kg), and the RPCAP contained a novel ruminally-protected capsaicin prill.
[2]TRT = main effect of treatment; CON vs CAP = contrast for treatments containing capsaicin (DCAP and RPCAP) vs CON.
[a,b,c]Means in rows with different superscript letters differ ($P < 0.05$).

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Effects of increasing sugar beets on steer backgrounding performance[1]

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ABSTRACT: The objective of this study was to evaluate the effects of sugar beets on steer backgrounding performance. Forty-eight Angus steers (260.7 ± 3.43 kg) were used in a completely randomized design for a 50 d study. On d -1, steers were weighed and assigned to 1 of 8 pens (6 steers per pen) equipped with GrowSafe units and one of four dietary treatments on d 0 (n = 12 steers/treatment; 2 pens/treatment: Table 1): 1) **0SB**: control diet with no sugar beets; 2) **15SB**: 15% sugar beets substituted for barley on a DM basis; 3) **30SB**: 30% sugar beets substituted for barley on a DM basis; and 4) **45SB**: 45% sugar beets substituted for barley on a DM basis. Sugar beets directly replaced rolled barley on a DM basis. All dietary treatments were formulated to meet or exceed the nutrient requirements of a 295 kg steer gaining 0.91 kg/d (NRC, 1996). The MIXED procedure of SAS was used for statistical analysis. Initial BW, mid-BW, final BW, period 1 and 2 ADG, and period 1 and 2 G:F were not different ($P \geq 0.33$) due to dietary treatment. There was also significant treatment × day interaction ($P < 0.001$) for DMI. On d 3, 19, 21, 23, 33, 44, and 45, 0SB DMI was reduced ($P \leq 0.05$), and increased ($P \leq 0.05$) on d 12, 20, and 47 compared with 15SB. On d 3, 19, 21, 33, 35, and 50, 0SB DMI was reduced ($P \leq 0.03$), and increased ($P \leq 0.01$) on d 9, 12, and 20 when compared with 30SB. On d 19, 21, 27, 33, 37, 38, and 45, 0SB DMI was reduced ($P \leq 0.05$), and increased ($P \leq 0.04$) on d 9, 24, and 35 when compared to 45SB. On d 35 and 37, 15SB DMI was reduced ($P \leq 0.002$), and increased ($P \leq 0.05$) on d 9 and 36 when compared with 30SB. On d 37 and 47, 15SB DMI was reduced ($P \leq 0.02$), and increased ($P \leq 0.03$) on d 1, 9, 44, and 46, when compared to 45SB. On d 45, 30SB DMI was reduced ($P \leq 0.03$), and increased ($P \leq 0.04$) on d 24 when compared to 45SB. These data suggest that backgrounding steers can be fed diets up to 45% sugar beets on a DM basis without negatively impacting performance.

Key words: backgrounding, steers, sugar beets

Introduction

Sugar beets are an excellent energy source, but are low in CP. Lardy and Schafer (2008) analyzed whole sugar beets with 6.8% CP and 81.0% TDN. Montana is a major producer of sugar beets in the Northern Great Plains (5th in the U.S.; USDA, 2015a), and excess or non-harvested sugar beets could provide a great alternative feedstuff for cattle Producers. In Montana, during the 2014-2015 sugar beet harvest, approximately 45.2 million pounds of sugar beets were not harvested (USDA, 2015b). This provides an excellent opportunity to sugar beet and livestock producers to utilize sugar beets as an alternative feed source. However, due to the moisture content of the sugar beets trucking and mileage need to be accounted for in the economic viability of feeding sugar beets.

Whole sugar beets are an excellent energy source (81% TDN; Lardy and Schafer, 2008), which could provide a potential replacement for traditional feedstuffs, such as barley or corn. However, care needs to be taken when feeding sugar beets to provide a crude protein source, sugar beets are a low-protein feedstuff (6.8% CP; Lardy and Schafer, 2008). Boucque et al. (1976) suggested that dried sugar beet pulp has a net energy value of 90% of that of barley and Arrizon et al. (2012) determined that dried shredded sugar beets has a net energy value of 82% of the value of steam-flaked corn. Based on the energy density and dry matter content (20.1% DM; Lardy and Schafer, 2008), sugar beets may make an excellent feedstuff in backgrounding rations for calves. Extra care needs to be taken when feeding sugar beets to cattle due to the increased potential as a choking hazard. Therefore, it is recommended that sugar beets be processed prior to feeding to minimize the choking risk to livestock. In addition, sugar beets contain sugar, which may improve feed intake and palatability of the diets, which is crucial for newly weaned beef calves starting a backgrounding diet.

We hypothesized that feeding increasing levels of sugar beets (0, 15, 30, and 45% of DM) would have no deleterious effects on steer feedlot growth, but would have improved palatability indicated by increased DMI as sugar beets increased in the diet. Therefore, the objective of this study was to evaluate the effects of sugar beets on steer backgrounding performance.

Materials and Methods

All procedures were approved by the animal care and use committee of Montana State University (#2015-AA09).

Animals and Diets. Forty-eight Angus steers (260.7 ± 3.43 kg) were used in a completely randomized design for a 50 d study. On d -1, steers were weighed and assigned to 1 of 8 pens (6 steers per pen) equipped with GrowSafe (GrowSafe Systems Ltd., Airdrie, AB Canada) units to allow for individual feed intake measurement. Steers were allotted into one of four dietary treatments on d 0 (n = 12 steers/treatment; 2 pens/treatment: Table 1): 1) **0SB**: control diet with no sugar beets; 2) **15SB**: 15% sugar beets substituted for barley on a DM basis; 3) **30SB**: 30% sugar

[1]Support for this research was provided by The Bair Ranch Foundation. The authors would also like to thank Brady Johnson, Maria Goettemoeller, Abbey Keyser, and Kate Perz for their assistance in conducting this trial.

Table 1. Ingredient and nutritional composition of diets fed to backgrounding steers (DM basis)

	Dietary Treatment[1]			
Item	0SB	15SB	30SB	45SB
Ingredient, %				
Sugar beets[2]	—	15.0	30.0	45.0
Rolled barley	45.0	30.0	15.0	—
Chopped hay	45.0	41.0	36.9	32.75
Soybean meal	6.25	10.40	14.75	19.0
Mineral premix[3]	0.90	0.90	0.90	0.90
Calcium carbonate	1.25	1.10	0.85	0.75
Salt	0.25	0.25	0.25	0.25
Deccox	1.35	1.35	1.35	1.35
Nutritional Composition[4]				
DM, %	87.4	74.4	64.7	57.3
TDN, %	66.6	65.5	64.5	63.4
CP, %	16.0	15.6	15.4	15.1
Ca:P	2.63	2.65	2.57	2.64

[1]Diets will be formulated to meet or exceed nutrient requirements of a 295 kg steer gaining 0.91 kg/d (NRC, 1996). Treatments were 0SB: 45% barley and 45% chopped hay; 15SB: 15% sugar beets substituted for barley on a % DM basis; 30SB: 30% sugar beets substituted for barley; and 45SB: 45% sugar beets substituted for barley.
[2]Sugar beets were processed through a wood chipper to reduce the particle size to reduce the risk of choking.
[3]Mineral premix: 13.6% Ca, 10% P, 15.6% salt, 1.0% Mg, 0.1% K, 2,500 mg/kg Cu, 35 mg/kg Se, 8,500 mg/kg Zn, 440,529 IU/kg vitamin A, 44,053 IU/kg vitamin D, and 881 IU/kg vitamin E.
[4]Calculated nutrient composition of the diets.

beets substituted for barley on a DM basis; and 4) **45SB**: 45% sugar beets substituted for barley on a DM basis. Sugar beets directly replaced rolled barley on a DM basis. All dietary treatments were formulated to meet or exceed the nutrient requirements of a 295 kg steer gaining 0.91 kg/d (NRC, 1996). Steers had continuous access to water and shelter. Steers were weighed on consecutive days on d -1 and 0, mid-point (d 26 and 27), and end (d 49 and 50) of the trial. Periods are defined as period 1 from d 1 to 27 and period 2 from d 28 to 50. Samples of the total mixed rations were collected weekly and dried in a forced air oven at 70°C for 48 h to determine DM. Individual DMI and G:F of each steer was calculated. Two steers were removed from the study due to non-treatment related illness. Sugar beets were processed through a commercial wood chipper to reduce the particle size.

Sampling and Laboratory Analysis. Ration samples were collected weekly composited by period (period 1: d 0 to 27; period 2: d 28 to 56). Blood samples were collected on d 0, 27, and 49 of the trial. Blood samples were collected from all calves via jugular venipuncture into 10-mL serum separator Vacutainer tubes. Blood samples were immediately be placed on ice for transport to the laboratory. Samples were centrifuged at 2,500 × *g* for 30 minutes. Serum was obtained and transferred to 5-mL polypropylene tubes and stored at -20°C for further analysis.

Statistical Analysis. The MIXED procedure of SAS will be used for the statistical analysis of all performance data. The dietary treatment was the fixed effect included in the model with a random effect of pen nested within treatment. Dry matter intake data was analyzed utilizing repeated measures with the fixed effects of dietary treatment, day, and the interaction. Four days of dry matter intake data were not calculated due to equipment failure. Individual animal is the experimental unit. Pre-planned comparisons of linear, quadratic, and cubic contrasts were utilized to partition treatment effects. Significance was determined at $P \leq 0.05$. To partition day effects and treatment × day interactions, LS Means was utilized ($P \leq 0.05$).

Results and Discussion

Initial body weights did not differ ($P = 1.00$; Table 2) by treatment design. Mid-point and final BW were not affected ($P \geq 0.63$). Overall ADG values were 1.38, 1.44, 1.55, and 1.58 ± 0.12 kg/d for 0SB, 15SB, 30SB, and 45SB treatments, respectively, and was not effected ($P \geq 0.55$) by treatment. These results were similar to Olfaz et al. (2005), which observed similar ADG in rams fed 40 or 60% sugar beet pulp. Results from this study suggest that the sheep fed diets with increased concentrations (60%) of sugar beet pulp gained more weight in similar time as sheep fed diets with 40% concentrations of sugar beet pulp. Although the relationship between treatment and ADG was insignificant in the current study, the ADG numerically increased with increasing concentrations of sugar beets in the diet. Arrizon et al. (2012) had similar results to the current study in that there was no significant relationship between ADG and dietary treatments with various concentrations of dried shredded sugar beets in the steer diets. The current study and previous studies suggest that whole sugar beets or sugar beet pulp may not impact steer body weight or ADG.

Overall G:F values were 0.189, 0.182, 0.200, 0.218 ± 0.016 for 0SB, 15SB, 30SB, and 45SB treatments, respectively, and was not effected ($P \geq 0.33$) by treatment. Contrary to the results in the current study, Arrizon et al. (2012) observed a linear decrease in feed efficiency as dried shredded sugar beets increased in the diet from 40 to 60%. Although we did not observe a decrease in feed efficiency, the divergent results between our study and Arrizon et al. (2012) may be due to differences in moisture content of the diet, which may have led to the difference in DMI.

Average daily DMI for the second period ($P = 0.10$) and overall ($P = 0.06$) tended to be effected quadratically by dietary treatment. There was also significant treatment × day interaction ($P < 0.001$; **Figure 1**) for DMI. On d 3, 19, 21, 23, 33, 44, and 45, 0SB DMI was reduced ($P \leq 0.05$), and increased ($P \leq 0.05$) on d 12, 20, and 47 compared with 15SB. On d 3, 19, 21, 33, 35, and 50, 0SB DMI was reduced ($P \leq 0.03$), and increased ($P \leq 0.01$) on d 9, 12, and 20 when compared with 30SB. On d 19, 21, 27, 33, 37, 38, and 45, 0SB DMI was reduced ($P \leq 0.05$), and increased ($P \leq 0.04$) on d 9, 24, and 35 when compared to 45SB. On d 35 and 37, 15SB DMI was reduced ($P \leq 0.002$), and increased ($P \leq 0.05$)

on d 9 and 36 when compared with 30SB. On d 37 and 47, 15SB DMI was reduced ($P \leq 0.02$), and increased ($P \leq 0.03$) on d 1, 9, 44, and 46, when compared to 45SB. On d 45, 30SB DMI was reduced ($P \leq 0.03$), and increased ($P \leq 0.04$) on d 24 when compared to 45SB. These results were similar to research conducted at NDSU that showed that including wet sugar beet pulp at concentrations greater than 20% of the diet will result in a reduction of DMI (Lardy et al., 2008). Olfaz et al. (2005) also observed similar results with decreases in DMI in rams when sugar beet pulp increased from 40% sugar beet pulp to 60% sugar beet pulp.

Implications

The current research suggests that whole sugar beets can replace barley up to 45% without negatively effecting performance. Further research is needed to find how increasing concentrations of sugar beet diets in backgrounding rations for steers effects meat quality.

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Table 2. Effects of increasing sugar beets on backgrounding performance of steer calves.

	Dietary Treatment[1]						Contrasts[2]		
Item	0SB	15SB	30SB	45SB	SEM	*P* – value	Linear	Quadratic	Cubic
BW, kg									
d 1	259.8	261.6	260.3	261.1	7.08	1.00	0.94	0.95	0.87
d 28	299.7	302.9	302.8	304.9	8.52	0.98	0.68	0.95	0.88
d 50	324.0	334.8	339.3	341.7	10.54	0.63	0.27	0.67	0.92
ADG, kg/d									
d 1 to 27	1.42	1.48	1.52	1.57	0.14	0.89	0.44	0.99	0.98
d 28 to 50	1.42	1.39	1.59	1.60	0.17	0.72	0.36	0.92	0.59
d 1 to 50	1.38	1.44	1.55	1.58	0.12	0.55	0.16	0.90	0.78
DMI, kg/d									
d 1 to 27	6.24	6.65	5.94	5.70	0.42	0.25	0.11	0.36	0.34
d 28 to 50	8.33	9.12	9.65	8.92	0.52	0.18	0.21	0.10	0.62
d 1 to 50	7.14	7.70	7.60	7.25	0.28	0.16	0.80	0.06	0.69
G:F									
d 1 to 27	0.23	0.22	0.24	0.27	0.02	0.41	0.20	0.27	0.79
d 28 to 50	0.16	0.15	0.17	0.18	0.02	0.72	0.43	0.59	0.64
d 1 to 50	0.19	0.18	0.20	0.22	0.02	0.33	0.12	0.41	0.71

[1]Diets will be formulated to meet or exceed nutrient requirements of a 295 kg steer gaining 0.91 kg/d (NRC, 1996). Treatments were 0SB: 45% barley and 45% chopped hay; 15SB: 15% sugar beets substituted for barley on a % DM basis; 30SB: 30% sugar beets substituted for barley; and 45SB: 45% sugar beets substituted for barley

[2]n = 12

[3]*P* -value for the *F*-test of the mean.

[4]*P*-value for linear, quadratic, and cubic effects of increasing sugar beets in the diet.

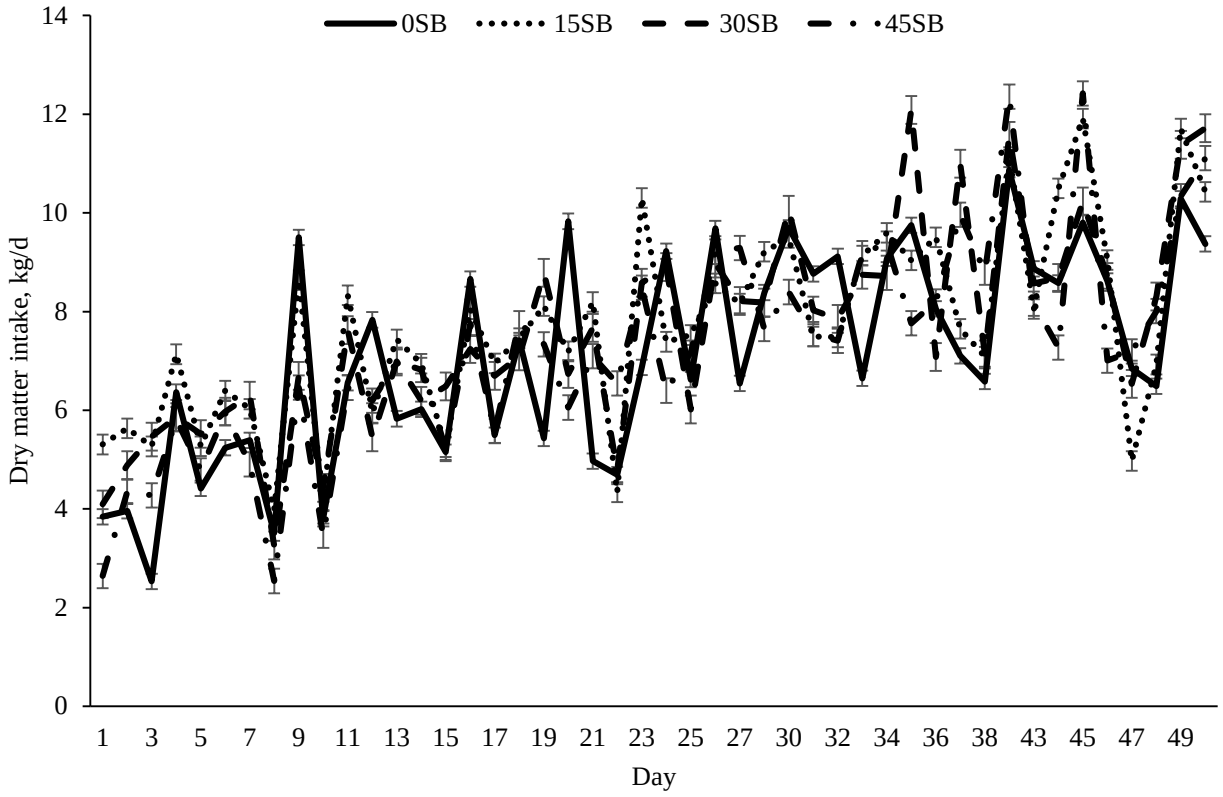

Figure 1. Effects of increasing sugar beets on steer dry matter intake. Diets were formulated to meet or exceed nutrient requirements of a 295 kg steer gaining 0.91 kg/d (NRC, 1996). Treatments were 0SB: 45% barley and 45% chopped hay; 15SB: 15% sugar beets substituted for barley on a % DM basis; 30SB: 30% sugar beets substituted for barley; and 45SB: 45% sugar beets substituted for barley. Dietary treatment: $P = 0.16$; day: $P = 0.14$; and dietary treatment × day: $P < 0.001$.

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Effect of crude protein supplementation on performance of cow-calf pairs and replacement heifers grazing late growing season forage

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ABSTRACT: Concurrent experiments were conducted to evaluate the effect of protein supplementation to beef cattle grazing warm-season shortgrass forage during the late growing season. Cattle in all experiments grazed adjacent shortgrass pastures dominated by Buffalograss (Buchloe dactyloides) and Blue Grama (Bouteloua gracilis). Stocking rates (≥ 2.3 ha / animal) were maintained such that forage availability was not limiting throughout the experiment. Precipitation in the area during the experiment was 176% of normal. For all Exp., treatments consisted of a supplemented group (1.32 kg per head of a 39% CP range cube fed 3 times a week) and a non-supplemented control group. Supplemented animals were fed a daily average of 0.22 kg of CP. In Exp. 1, 45 multiparous cow-calf pairs (initial BW 646 ± 13 kg) were individually weighed and body condition scored every 14 d. Forage clippings were taken simultaneously with BW measurements. Cow measurements and forage clippings began July 6 and concluded September 28. Cow final BW (P = 0.24) and ADG (P = 0.38) were not affected by treatment. There was no difference (P = 0.97) in cow final BCS regardless of treatment. Calf ADG (P = 0.54) and weaning weight (P = 0.45) were not affected by treatment. In Exp. 2, 26 primiparous cows (initial BW 546 ± 12 kg) were supplemented and measurements obtained in the same manner as Exp.1. Cow final BW (P = 0.39) and final BCS (P = 0.81) did not differ between treatments. Cow ADG (P = 0.07) tended to be greater when supplemented with 0.22 kg CP per day. Calf ADG (P = 0.50) and weaning weight (P = 0.11) did not differ between treatments. In Exp. 3, 25 replacement heifers (initial BW 412 ± 9 kg) were observed for BW and forage clippings were obtained every 14 d. Heifer final BW (P = 0.17) was not different between treatments. Heifer ADG (P = 0.02) was greater for supplemented heifers. Supplementing protein to cattle grazing late season medium quality forage is advantageous for increasing ADG in replacement heifers and potentially beneficial to improve condition in lactating primiparous cows. Repeating this experiment under varied precipitation patterns, as is normal for short-grass regions, would be beneficial to further examine the impact of late growing season protein supplementation on cow-calf pair/replacement heifer performance.

Keywords: Beef cows, forage quality, supplementation

INTRODUCTION

Providing a CP supplement to ruminants grazing low quality (CP < 7%) forage generally increases forage intake (McCollum et al., 1985). Pitts et al. (1992) reported that steers grazing warm season shortgrass prairie exhibited greater weight gain during the summer (April – July) when provided a CP (0.14 or 0.28 kg / d) supplement. In young growing forages, CP is at its greatest while fiber is low; however, as the plant matures CP begins to decrease and fiber begins to increase (Van Soest, 1994). This shift occurs as the maturing plants draws carbohydrates from below-ground stores to use for energy, replacing CP. Low quality forages, lacking in CP concentration, do not provide sufficient N to rumen microbes in cattle which is necessary to breakdown forage (Satter and Slyter, 1974). Several studies (McCollum et al., 1985; Pitts et al., 1992) have demonstrated supplementing protein to yearling cattle grazing low quality forage improves intake and performance. Limited research exists which observes the effect supplemental protein on performance in cow-calf pairs grazing late season forage.

Greater milk yields have been reported from cattle given supplemental protein while grazing low-quality forage (Forcherio et al., 1995). According to Rutledge et al. (1971) the single most important determinant of weaning weight is the lactation performance of the dam. Increased weaning weights would offset the cost of supplementation and provide additional income for the producer. In the current experiment, it was expected that if nutrient availability to dams is increased under low quality forage conditions, calf performance will increase. The objective of these experiments was to determine the effect of protein supplementation to cattle grazing during late growing season at increasing performance in calves pre-weaning, maintaining condition of dams, and improving replacement heifer performance.

MATERIALS AND METHODS

All procedures were approved by the West Texas A&M University/CREET Institutional Animal Care and Use Committee. The experiments began July 6th and continued for 12 weeks until calf weaning, September 28. All experiments were conducted at West Texas A&M University Nance Ranch near Canyon Texas. Precipitation from May 1 through October 1 was 176% of normal (Western Regional Climate Center; Mean precipitation calculated using values observed from 1948-2013).

Experiment 1. British cross multiparous cows (n = 45; initial BW 646 ± 13 kg; age 5.5 ± 1.8 years) were stratified by initial body weight and randomly assigned to a supplemented treatment (**TRT**, 1.32 kg of a 39% CP fed 3 times a week), or a non-supplemented control. Calves nursed cows for the entirety of the experiment. Calves were 85 ± 23 days old and had an initial BW of 137 ± 8 kg at onset of the experiment. Cattle receiving the crude protein supplement received 0.22 kg of CP per day. Supplement level was based on Oklahoma State University's Oklahoma Gold supplement program (Lalman and Gill, 1999). Oklahoma Gold reports increased

Table 1. Analysis of range cubes[1]

Nutrient	%, DM basis
CP[2]	39.0
Crude Fat[3]	2.3
Crude Fiber[3]	14.0

[1]HI-PRO, Rancher Pro 38; Cottonseed meal based supplement
[2]Nutrient analysis conducted by commercial laboratory (Servi-Tech Laboratories, Amarillo, TX)
[3]Analysis provided by HI-PRO Feeds (Friona, TX)

performance of stocker cattle grazing late-season forage from feeding 0.45 kg of a 38-41% CP supplement daily (Lalman and Gill, 1999).

All cattle grazed a single shortgrass prairie pasture (164.7 ha), and were managed as a single group for the duration of the experiment. Cattle in Exp. 1 were stocked at a rate of 3.66 ha / cow-calf pair. The shortgrass pasture was dominated by Buffalograss (*Buchloe dactyloides*) and Blue Grama (*Bouteloua gracilis*).

Supplements were fed during the 12 week period preceding calf weaning (September 28). Feeding commenced on July 6 and concluded when calves were weaned. Supplements were fed to cattle at 0700 three mornings a week. On mornings that cattle were supplemented all cattle were brought in to a sorting facility and sorted by TRT or CON. Cattle receiving no supplement were immediately returned to pasture along with all calves. Cattle receiving supplement were penned individually and fed 1.32 kg (DMB) of a 39% crude protein range cube. The cattle were allotted 1 hour to consume the supplement. No ORTS were collected due to all cattle consuming the entirety of supplement at each feeding. Upon completion of consuming the cubes the treatment cattle were let back out to pasture.

At onset of the experiment, and again at weaning, cows and calves were weighed and cows were also evaluated for body condition (1-9 scale; Herd et al., 1986) for 2 consecutive days to obtain an average initial body weight and final body weight. Body condition scores were taken by the same 2 trained technicians each time. Cattle (cows and calves) were weighed every 14 d during the experiment. Prior to weigh days cattle were collected shortly before nightfall. Cows and calves had *ad-libitum* access to prairie hay and water all night before being weighed at 0600. Upon conclusion of weight and BCS collection cattle were moved back to their pasture, sorted, and treatment cattle were fed supplement. Forage clippings were taken from the pasture every 14 d, corresponding with weigh days. A clipping square that measured 0.732 m^2 was used to take 10 clippings. Forage was hand clipped to ground level and bagged. Forage availability was calculated: dried forage, g * 44.85 = kg of dried forage / ha (USDA, 2006).

Experiment 2. British cross primiparous beef cows (n = 26; initial BW 546 ± 12 kg; 2 years old) were stratified by initial body weight and randomly assigned to supplemented treatment (TRT, 1.32 kg of a 39% CP fed 3 times a week), or a non-supplemented control. Calves nursed cows for the entirety of the experiment. Calves were 103 ± 17 days old and had an initial BW of 137 ± 5.4 kg at onset of the experiment. All cattle grazed a single mixed shortgrass prairie pasture (192.2-ha) and were managed as a single group for the duration of the experiment. The shortgrass pasture was dominated by Buffalograss (*Buchloe dactyloides*) and Blue Grama (*Bouteloua gracilis*). Cattle in Exp. 2 were stocked at a rate of 7.39 ha / cow-calf pair. No ORTS were collected due to all cattle consuming the entirety of supplement at each feeding. Primiparous cows were supplemented and measurements obtained in the same manner as Exp.1.

Experiment 3. British cross yearling heifers (n = 25, initial BW 412 ± 9 kg) were stratified by initial body weight and randomly assigned to supplemented treatment (TRT, 1.32 kg of a 39% CP fed 3 times a week), or a non-supplemented control. All heifers grazed a single mixed shortgrass prairie pasture (57.5-ha) for the duration of the experiment. Heifers in Exp. 3 were stocked at a rate of 2.3 ha / animal. The shortgrass pasture was dominated by Buffalo Grass (*Buchloe dactyloides*) and Blue Gramma (*Bouteloua gracilis*). No ORTS were collected due to all cattle consuming the entirety of supplement at each feeding. Replacement heifers were supplemented and measurements obtained in the same manner as Exp.1 and 2.

Dry matter of forage clippings were analyzed by drying samples at 55°C in a forced-air oven for 48 hours. Samples were then composited within period and pasture and submitted to Servi-Tech (Amarillo, TX, USA) for analysis of CP, NDF, ADF, and TDN.

Body weight and ADG were analyzed as a linear mixed model with one-way treatment structure in a completely randomized design (PROC MIXED; SAS Institute Inc., Cary, NC), with animal serving as the experimental unit. The class statement treatment and the model statement included treatment.

Body weight change overtime was analyzed as repeated measures in a completely randomized design (PROC MIXED; SAS Institute Inc., Cary, NC), with animal serving as the experimental unit. The class statement included treatment and period and the model statement included treatment, period, & period*trt interaction.

Means separation and P-values were determined using LSMEANS with PDIFF option. Treatment differences are discussed when $P \leq 0.05$; tendencies are discussed when $P > 0.05$ and < 0.10.

RESULTS AND DISCUSSION

Experiment 1 (Mature Cows)

Forage analysis and available forage are in summarized in Table 2. Beef cattle forage intake is maximized when approximately 0.04 kg of forage mass / kg of live animal weight is available; however intake is thought to depressed when 0.02 kg forage mass/ kg of live animal weight is realized (NRC, 1987). Intake should not have been depressed as forage mass remained well above critical limits (Table 2). Cow final BW, ADG, and final BCS ($P \geq 0.24$) were not affected by treatment.

Moore et al. (1999) reported that under conditions where TDN:CP ratio > 7:1 protein supplementation increased voluntary feed intake. Greater forage digestion and ruminal passage rate are the mechanisms behind a positive response to protein supplementation (McCollum et al., 1985; Koster

et al., 1996). Greater ruminal passage rate would provide greater nutrient flow to the lower GI which would increase animal performance. TDN:CP ratio in this experiment ranged from 6.5:1 to 8.1:1 (Table 2). TDN:CP ratio increases as the CP content of forages decreases in late season forage (Moore et al., 1999). A TDN:CP > 7:1 is not uncommon during the late growing season. While Moore et al. (1999) reported increased performance when TDN:CP > 7, inconsistency of forage quality in the current experiment made differences associated with the TDN:CP ratio negligible.

Calf ADG (P = 0.54) was not affected by treatment. Forcherio et al. (1995) fed lactating beef cows grazing tall fescue two different levels (100 g/d or 200 g/d) of CP from late May until late July. Calves from dams receiving CP regardless of level tended to have increased milk intake when compared to calves whose dams received no supplement. They were unable, however to conclude that calves from supplemented dams performed significantly greater than their non-supplemented counterparts..

No difference (P = 0.45) was observed in weaning weights of calves between treatment. Beaty et al. (1994) supplemented mature beef cows grazing tall grass pastures four different levels of protein starting prepartum through calf weaning. Cattle grazed tallgrass pastures. Forage quality varied as the experiment started 105 d before calving and continued until calf weaning (321 d total). There was a linear relationship between level of protein fed and weaning weight, as weaning weight increased when level of protein fed increased.

Variation in animal and forage characteristics has a great impact as to the efficacy of supplemental protein (Mathis et al., 2000). Cattle grazing native range pastures will have different responses to supplemental protein than cattle grazing monocultures as seen in the current experiment and other studies (Beaty et al., 1994; Forcherio et al., 1995). Cattle in this experiment were stocked at a very light stocking rate and had abundant opportunity to select a diet more nutritious (Coleman et al., 1973) than that of the reported forage average.

Experiment 2 (First-calf Heifers)

Forage analysis and available forage are summarized in Table 3. As in Exp. 1 available forage was maintained above a level (0.04 kg forage / kg live animal weight; NRC, 1987) that would not depress intake. Differences were not observed (P = 0.39) in cow final BW between treatments. Cow ADG tended (P = 0.07) to be greater in the TRT-group. However there was no difference (P = 0.81) in cow BCS.

Tendencies for TRT ADG to be greater would plausibly be attributed to the age of the primiparious cows used, as they were just two years old. Primiparous cows only reach ~80% of mature size at 24 months of age. If parturition is planned to take place at this age then the animal must have adequate nutrition for lactation and growth (Freetly et al., 2006). Growth stimulation could indicate that supplemental protein improved the nutritional status of the animal. Cows at this stage of production have many nutritional challenges, first lactation, second gestation, and continued growth (Johnson and Funston, 2013).

No differences (P = 0.11) were found in calf weaning weight. Rutledge et al. (1971) reported that 60% of the variance in 205 d weaning weights could attributed to differences in milk yield. Age of the dam was most closely related to milk production as it has a quadratic relationship with age. Milk yield peaked when cows were approximately 8.4 years of age. We hypothesized that supplement may increase milk production in primparous cows, and the difference would manifest as increased calf weight. However, this was not observed.

A TRT x Pd interaction (P = 0.017; Figure 2) was seen in calf ADG. In periods 1-5 inconsistency of calf performance in relation to treatment is due to forage quality. Using the TDN:CP ratio (Moore et al., 1999) described prior, forage quality in periods 1-5 varied from a high of 8.2:1 to 6.8:1. In the sixth period however the TDN:CP ratio was 9.7:1. This could suggest that in earlier periods forage quality was not limiting milk production as the animals were choosing a diet more nutritious than reported. However, in the sixth period primiparous cows would have been challenged to choose a nutritionally adequate diet. Supplemental protein may have increased TRT cow intake, which in turn may increase milk yield, subsequently increasing TRT calf performance in the sixth period.

Experiment 3 (Replacement Heifers)

Forage analysis and available forage is shown in Table 5. Available forage did not reach a level that would have decreased forage intake. No differences (P = 0.17) were found in final BW of heifers. Heifers receiving CP supplement did (P = 0.02) have a greater ADG than CON heifers.

Protein supplementation during the late growing season on low quality forages has been reported to increase performance in steers (McCollum et al., 1985; Pitts et al., 1992). Under the conditions of this experiment, supplemental protein proved to increase ADG in replacement heifers. Increased performance of replacement heifers in late growing season would lead to better condition going into winter. Heifers entering winter in good condition could benefit as they would be better able to maintain condition through gestation. Improving condition of gestating heifers shortened rebreed time. (Bagley,1993). Inadequate size at first parturition limits milk production and conception during lactation (NRC, 1996).

IMPLICATIONS

Under the conditions of this experiment calf performance from either primiparous cows or multiparous cows increased in response to supplementation. This is attributable to the quality of forage grazed. In subsequent studies it would be advantageous to quantify milk yield from primiparous and multiparous cows being supplemented protein while grazing forage of varying quality. Differences in ADG of young beef females grazing medium quality forage prove the ability of supplemental protein to improve body condition in young beef females entering winter. Abnormal temperatures and precipitation patterns may have predicated the abnormal forage growth patterns. Due to the inherent variability of precipitation patterns in shortgrass prairie, we intend to repeat this experiment to evaluate the effect of supplemental protein during the late growing season on cow, calf, and replacement heifer weight gain under varying climatic conditions.

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Table 2. Chemical composition and availability of forage grazed in Exp. 1

	Period					
Nutrient Analysis[1], DM basis	1	2	3	4	5	6
CP, %	8.0	6.9	8.1	7.3	6.3	6.2
ADF, %	43.8	43.5	45.1	49.2	47.7	46.7
TDN, %[2]	53.4	54.3	52.5	47.1	48.9	50.7
Forage available[3]	2.43	2.36	2.41	2.01	2.18	2.37

[1]Nutrient analysis conducted by commercial laboratory (Servi-Tech Laboratories, Amarillo, TX).
[2]NRAES-63. Penn State Univ. Dairy Reference Manual. 1995. Table 5.25, p 108
[3]Forage available = kg forage / kg live animal weight. (Sollenberger et al., 2005)

Table 3. Effect of protein supplementation on cow and calf performance (Exp. 1)

	Control	Treatment[1]	SEM	P-value
Cow				
Initial BW, kg	654	639	13.0	0.41
Final BW, kg	678	658	11.8	0.24
ADG, kg	0.28	0.23	0.04	0.38
Initial BCS[2]	6.07	5.89	0.14	0.37
Final BCS[2]	6.16	6.15	0.13	0.97
Calf[3]				
Initial BW, kg	144	130	8.0	0.24
Weaning weight, kg	243	233	9.3	0.45
ADG, kg	1.19	1.22	0.03	0.54

[1]Treatment group received 1.32 kg of a 39% CP range cube 3 times a week
[2] 1-9 scale; Herd et al., 1986
[3]1 Calf died during experiment, not related to dietary treatment

Figure 2. The effect of supplemental protein on calf ADG by period from primiparous cows grazing shortgrass prairie during the late growing season; Exp. 2; TRT x Pd (P = 0.02); SEM = 0.06.

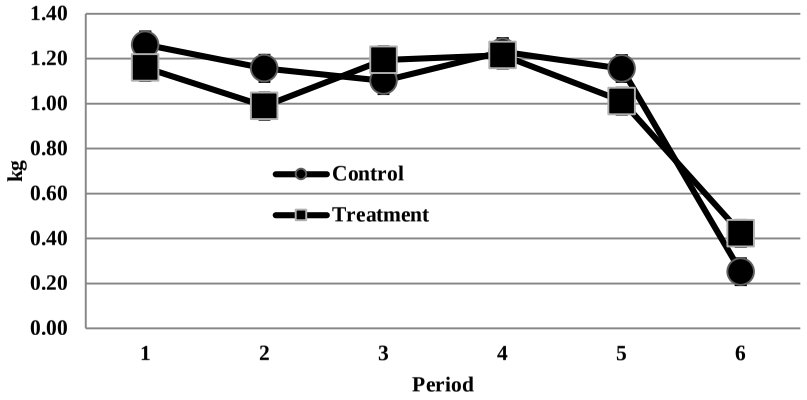


Table 4. Chemical composition and availability of forage grazed in Exp. 2

	Period					
Nutrient Analysis[1], DM basis	1	2	3	4	5	6
CP, %	6.5	7.4	6.6	7.0	6.0	5.3
ADF, %	44.1	44.6	47.0	48.9	51.7	45.8
TDN, %[2]	53.4	52.5	49.8	48.0	45.3	51.6
Forage available[3]	6.16	6.67	6.05	6.28	4.08	5.17

[1]Nutrient analysis conducted by commercial laboratory (Servi-Tech Laboratories, Amarillo, TX)
[2]NRAES-63. Penn State Univ. Dairy Reference Manual. 1995. Table 5.25, p 108
[3]Forage available = kg forage / kg live animal weight. (Sollenberger et al., 2005)

Table 5. Effect of protein supplementation on primiparous cow and calf performance (Exp. 2)

	Control	Treatment[1]	SEM	P-value
Cow				
Initial BW, kg	543	548	11.6	0.76
Final BW, kg	547	561	11.8	0.39
ADG, kg	0.04	0.15	0.04	0.07
Initial BCS[2]	5.88	5.88	0.12	1.00
Final BCS[2]	5.81	5.85	0.11	0.81
Calf				
Initial BW, kg	143	132	5.4	0.16
Weaning weight, kg	230	217	5.7	0.11
ADG, kg	1.04	1.01	0.03	0.50

[1]Treatment group received 1.32 kg of a 39% CP range cube 3 times a week
[2]1-9 scale; Herd et al., 1986

Table 6. Chemical composition and availability of forage grazed in Exp. 3

	Period					
Nutrient Analysis[1], DM basis	1	2	3	4	5	6
CP, %	6.4	7.5	6.8	6.9	5.8	5.2
ADF, %	44.2	44.1	44.6	46.2	46.1	44.9
TDN, %[2]	53.4	53.4	52.5	50.7	50.7	52.5
Forage available[3]	2.94	1.93	1.71	1.85	1.79	2.44

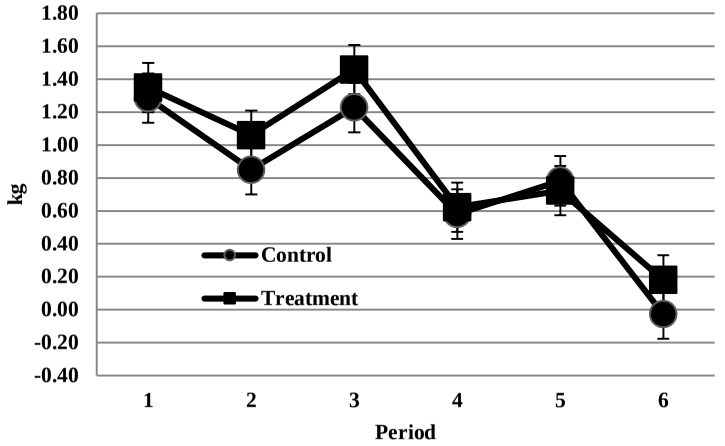
[1]Nutrient analysis conducted by commercial laboratory (Servi-Tech Laboratories, Amarillo, TX)
[2]NRAES-63. Penn State Univ. Dairy Reference Manual. 1995. Table 5.25, p 108
[3]Forage available = kg forage / kg live animal weight. (Sollenberger et al., 2005)

Table 7. Effect of protein supplementation on yearling heifer performance (Exp. 3)

	Control	Treatment[1]	SEM	P-value
Initial BW, kg	407	416	8.9	0.52
Final BW, kg	475	492	9.0	0.17
ADG, kg	0.80	0.91	0.03	0.02

[1]Treatment group received 1.32 kg of a 39% CP range cube 3 times a week

Figure 7. The effect of supplemental protein on ADG by period in yearling heifers grazing shortgrass prairie during late growing season; Exp. 3; TRT x Pd (P = 0.91); SEM = 0.15.



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Effect of corn-based supplementation on gas emissions, performance, and energetic losses of steers grazing wheat pasture

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ABSTRACT: Thirteen Angus-cross steers (initial BW = 436 ± 24 kg) were used in a crossover design to evaluate the effects of corn supplementation on gas emissions, performance, and energetic losses of steers grazing wheat pasture. Steers were allowed *ad libitum* access to wheat pasture (1.2 steers/ha), and were individually supplemented one of two treatments daily for two 30 d periods. Treatments included either 0.2 kg of pelleted wheat middlings (**CON**), or a dry-rolled corn supplement fed at 0.5% of BW plus 0.2 kg of pelleted wheat middlings (**SUPP**). After initial 30 d period, treatments were alternated and steers were supplemented an additional 30 d. Fecal output was determined with titanium dioxide (**TiO_2**) as an external marker. Beginning on d 14 of each period 15 g of TiO_2 was added to each steers supplement. In vitro analysis of wheat forage was determined to estimate DM digestibility of the wheat forage for each 30 d period. Forage intake was calculated using the determined fecal output and estimated forage digestibility. Ruminal CH_4 and CO_2 fluxes were measured using a GreenFeed (C-Lock Inc., Rapid City, SD) system. Urine energy loss was assumed to be 1.4% of GE intake. Oxygen production was estimated from CO_2 production, assuming a respiratory quotient of 1.05. Forage intake as percent of BW did not differ (P = 0.15) between CON (3.22%) and SUPP (3.61%). Average daily gain for CON and the SUPP averaged 1.4 kg and 1.3 kg, respectively, and was not influenced (P = 0.54) by supplementation. There were no differences ($P \geq 0.63$) among treatments for OM digestibility (CON: 84.9%; SUPP: 84.6%) and NDF digestibility (CON: 82.5%; SUPP: 83.1%). Carbon dioxide excreted (CON: 9.8 kg/d; SUPP: 10.5 kg/d) tended to be less (P = 0.08) for CON. No differences (P = 0.43) were observed in CH_4 emissions among CON and the SUPP supplement (334 and 351 g CH_4/d, respectively). Corn supplementation decreased (P = 0.02) CH_4 g/kg of DMI by 20.5%. Methane as percent of GE intake was decreased (P = 0.02) by 21.6% when steers consumed the SUPP compared to CON. Heat production as a percent of GE intake decreased (P = 0.03) when steers consumed the SUPP. Under the conditions of this experiment, cereal grain supplementation reduced CH_4 emissions.

Keywords: methane, wheat pasture, energetic losses

INTRODUCTION

Greenhouse gas (**GHG**) emissions are becoming of increasing importance to livestock producers, with greater scrutiny from regulatory bodies and consumer perspective. Methane production represents an energetic loss of fermentation in the rumen of cattle. Cattle consuming forages compared to cattle consuming high concentrate diets produce greater amounts of CH_4 g/kg of DMI (Johnson and Johnson, 1995). Millions of cattle graze wheat pasture each year in the United States (Horn et al., 1977), because wheat forage is a dual-purpose crop that provides livestock economical gains without a reduction in grain production. Whole-body calorimeters are the traditional method of measuring gaseous emissions of ruminants. Nevertheless, whole-body calorimeters do not account for environmental factors of cattle in production settings. Sulfur hexafluoride (**SF_6**) is a method of measuring CH_4 emissions in a production setting, but SF_6 is a laborious and invasive procedure (Hammond et al., 2013). The GreenFeed (**GF**, C-Lock Inc., Rapid City, S.D.) system is an automated headbox that measures carbon dioxide (**CO_2**) and methane emissions (**CH_4**). Feed automatically dispenses to entice steer to use the unit. It is considered a less invasive system relative to whole-body calorimeters and SF_6 systems (Hammond et al., 2013).

Few experiments have studied the effects of corn supplementation to cattle grazing and the CH_4 mitigation effects concentrates impose. With supplementation of grain to cattle, the acetate:propionate ratio may be decreased, and cause a shift towards more energetic efficient microbes in the rumen reducing CH_4 production (Hristov et al., 2013). The purpose of the current experiment was to establish modern baseline gas emissions of cattle grazing wheat pasture with a noninvasive automated headbox to measure GHG emissions, and research the effects of corn supplementation on CH_4 emissions of cattle grazing wheat pasture.

MATERIALS AND METHODS

All procedures involving the use of animals were approved by the West Texas A&M University Institutional Animal Care and Use Committee (approval number 41014).

Thirteen Angus-cross steers (initial BW = 417 ± 13 kg) were used in a crossover design to determine the effects of concentrate supplementation on greenhouse gas emissions while steers grazed wheat pasture from March 8 to May 7, 2015. Steers were weighed two consecutive days (d0 and d1) to minimize effects of gut fill. Steers were randomly assigned to 1 of 2 treatments; 0.2 kg of pelleted wheat middlings (**CON**) or 0.2 kg pelleted wheat middlings with a corn-based supplement fed at 0.5% of BW (**SUPP**). The CON treatment was provided to dose steers with the external marker and establish equal daily handling of steers. The experiment consisted of two 30 day periods (Period 1: March 8 to April

7; Period 2: April 8 to May 7, 2015). After the first 30 day period, steers were alternated to the opposite treatment. Steers were allowed *ad libitum* access to 8.3 ha of wheat pasture (*Triticum aestiuum)* for 23 h/d with the remaining hour used for treatment supplementation. Steers were given access to an additional 2.7 ha in the second period to ensure *ad libitum* access of wheat forage because of concerns with lack of precipitation decreasing forage availability. Forage availability before experiment equaled 1,344 kg/ha, and after first period forage availability equaled 1,130 kg/ha. After addition of the 2.7 ha, forage availability increased to 1376 kg/ha. Steers were gathered each day at 1100, and sorted into 13 individual pens. Steers were allotted 1 hour to consume supplement, and orts were collected. Treatments were readily consumed, and only one steer left a measurable amount of orts during the collection period. All steers had access to a free choice mineral with magnesium oxide (10% magnesium oxide; Hi-Pro Feeds, Friona, TX) and access to a mineral block containing polaxalene (Sweetlix Livestock Supplement System, Mankato, MN) to mitigate frothy bloat. Mineral block was formulated to administer 15 g of polaxalene/d.

Table 1. Composition of wheat forage

Item, % DMB	Period 1[1]	Period 2[1]
DM	39.7	36.6
CP	20.9	15.1
NDF	37.3	40.5
ADF	22.0	25.4
IVDMD	84.4	78.4

[1]Period 1 (March 8 to April 7) Period 2 (April 8 to May 7)

Titanium dioxide was used as an external marker to estimate fecal output (Titgemeyer et al., 2001). Titanium dioxide was mixed with the pelleted wheat middlings and was provided to deliver 15 g of TiO_2 per head/d for 14 days leading up to each collection and the six days during each collection. Orts were collected and analyzed for residual TiO_2.

Fecal collections were taken twice daily for six consecutive days starting on d 24 of each period to determine digestibility and energetic losses. Time was advanced 2 hours every 24 hours to minimize effects of diurnal variation. Steers were gathered, walked to a working chute on site, and rectally palpated for a fecal sample.

During the experiment, steers had access to a GreenFeed unit, which measures CO_2 and CH_4 emissions (Hammond et al. 2013). The GF system measures a 3 to 5 minute spot sample of CH_4 and CO_2 the steer excretes. Exhaled gases are pulled through a tube under negative pressure where airflow mass is measured continuously. Measurement of CH_4 and CO_2 concentration are analyzed using on-board nondispersive infrared sensors.

Laboratory Analyses

Dry matter of feces, feed, and orts were analyzed by drying samples to a constant weight at 55° C in a forced-air oven for 48 hours. Wheat forage samples were taken daily during each collection period. Samples were then ground through a 1mm screen using a Wiley Mill (Model 4, Thomas-Wiley, Philadelphia, PA). Lab corrected DM on fecal, ort, and supplement samples were conducted with forced-air oven at 105° C for 24 hours. Ash content was determined by combusting fecal, ort, and supplement samples in a muffle-furnace at 450° C for 8 hours. Gross energy of feces and feed were determined using an automatic bomb calorimeter (Parr 6400 Calorimeter, Parr Instrument Company, Moline, IL). Total N of feed and feces were determined by combustion in a C/N analyzer (Elementar C/N Vario Max Cube, Elementar Americas Inc., Mt. Laurel, NJ). Starch content of feed and feces was determined using an enzyme kit (Megazyme International Ireland Ltd., Wicklow, Ireland; method 996.11; AOAC, 2003).

Table 2. Composition of supplements

Ingredient, % DMB	CON[1]	SUPP[1]
Pelleted wheat middlings	87.1	30.7
Dry-rolled corn	0.0	65.5
Molasses	4.6	3.8
TiO_2	8.3	0.0
Analyzed nutrient composition, DMB		
Starch, %	18.6	51.3
CP, %	17.0	11.6

[1]CON = 0.2 kg of pelleted wheat middlings; SUPP = supplemented 0.5% BW of corn-based supplement plus 0.2 kg of pelleted wheat middlings.

Concentration of TiO_2 in fecal and ort samples were determined using a spectrophotometer (Synergy 2, Biotek, Winooski, VT) using the method described by Myers et al. (2004). A blank fecal background was used to correct for baseline TiO_2 in fecal samples (Morgan et al., 2014). A fecal background sample was collected from steers prior to TiO_2 dosing.

To determine wheat forage digestibility, wheat forage was ground to 1mm and 1g samples were weighed into F57 filter bags (Ankom Technology, Macedon, NY), and incubated in 250 ml bottles for 48 h at 39° C using an in vitro gas production system (Ankom Technology, Macedon, NY). Wheat samples were analyzed in triplicate with triplicate blanks as a correction. Rumen fluid was used from two steers consuming a ration in excess of 50% forage. Rumen fluid was strained through four layers of cheesecloth after collection. A 2:1 McDougall's buffer:ruminal fluid was used for the incubation. A subsequent NDF analysis on the filter bags was used to determine true IVDMD.

Calculations

Dry matter intake of wheat forage was calculated by dividing fecal output by in vitro DMD and then subtracting the known intakes of the supplements (Merchen, 1988).

Using CH_4 and CO_2 estimated from the GF unit, assuming urine energy at 1.4% of GE intake (unpublished data), and a respiratory quotient of 1.05, heat production was calculated using the equation of Brouwer (1965). Recent reports, using whole-body calorimetry chambers (Hales et al., 2012) and head boxes (Thornton and Owens, 1981) suggest an RQ near or less than 1.0. The RQ value of 1.05 was chosen because by definition, an RQ of greater than 1.0

suggests the accretion of fat, and given the physiological stage of the steers, fat would be deposited greater than protein. While the possibility exists for RQ to vary by treatment, other calorimetry research has not reported this (Hales et al., 2012). Urinary energy was calculated as the quotient of gross heat (2.3 kcal/g) of urea assuming all urinary N was urea (Street et al., 1964).

Statistical Analysis

Growth performance and gaseous emissions were analyzed as a completely randomized design (PROC MIXED; SAS Institute Inc., Cary, NC) as a mixed model with one-way treatment structure, with animal serving as the experimental unit. The class statement included steer and period. The model statement included treatment, period, and treatment *x* period. No interactions between treatment and period were detected ($P > 0.10$); therefore main effects of treatment were reported. Means separation and *P*-values were determined using LSMEANS. Treatment differences were discussed when $P \leq 0.05$; tendencies were discussed when $P > 0.05$ and < 0.10.

RESULTS AND DISCUSSION

Intake and Digestibility

Steers provided supplement had greater ($P < 0.01$; Table 3) DMI (forage and supplement) than non-supplemented steers. Forage intake was not influenced ($P = 0.13$) by SUPP, and forage intake as a percent of BW was not influenced ($P = 0.15$) by SUPP. Intake estimates in the current experiment agree with forage intake estimations from other experiments (Branine and Galyean, 1990). Branine and Galyean (1990) supplemented cattle grazing wheat pasture (> 20% CP) with 0.5 kg of a steam-flaked milo and observed no differences in intake between cattle consuming the supplement and cattle consuming no supplement. Branine and Galyean (1990) offered one third of the grain relative to the grain offered in the current experiment. Judkins et al. (1997) supplemented steers, grazing fescue pasture (15% CP), ground corn 0.4% of BW, and did not observe a reduction in forage intake compared to steers not supplemented. Traditionally, concentrate supplementation to ruminants consuming forage is reported to cause decreased forage intake and decreased forage digestibility (Horn and McCollum, 1987). However, the negative associative effect of concentrate supplementation and grazed forage is typically observed with consumption of low-quality forage. Forage with concentrations of CP above 15% may eliminate the negative association effects of concentrate supplementation of steers consuming forage. Moore et al. (1999) describes a TDN:CP of the diet above 7 as the threshold for decreased forage intake. The CP content of the wheat (15% or greater) in the current experiment and other experiments may cause a large enough "surplus" of CP that supplemented concentrate did not increase the TDN:CP above seven. Olson et al. (1999) ruminally dosed increasing levels of starch concurrently with increasing levels of rumen degradable protein (**RDP**) to steers consuming low-quality forage. Starch decreased forage intake as expected, but increasing levels of RDP mitigated the intake effects of the starch.

Organic matter digestibility and NDF digestibility were not affected ($P \geq 0.47$) by SUPP. Forage digestibility is a concern when supplementing forage diets with a concentrate, as the reduction in pH from rapid fermentation of concentrates produced in the rumen is harmful for cellulolytic species (Horn and McCollum, 1987). Hess et al. (1996) supplemented steam-flaked corn at 0.34% of BW while ruminally-cannulated steers grazed endophyte-free fescue, and reported no differences in NDF digestibility or OM digestibility after an in situ analysis for 96 h. Judkins et al. (1997) reported no differences in NDF and OM digestibility when supplementing steers grazing fescue with ground corn at 0.4% of BW. Elizalde et al. (1998) supplemented dry-rolled corn (1.4 kg/d) to steers grazing native tall fescue (>20% CP), and reported no differences in NDF and OM digestibility between steers receiving corn supplement and steers not receiving supplement. The lack of differences in the current experiment of NDF and OM digestibility may be explained by the "surplus" of dietary CP provided by forage CP as explained above. Olson et al. (1999) reported tendencies for CP to mitigate starch effects on digestibility, similar to the intake effects of starch.

Klevesahl et al. (2003) ruminally dosed increasing levels of starch concurrently with increasing levels of RDP to steers consuming low-quality forage, and reported decreased NDF digestibility with increasing starch. However, rumen pH of steers was not less than 6.3 among treatments, indicating no effect of pH on fiber digestion. Olson et al. (1999) reported differences in fiber digestion among treatments, but pH was not less than 6.25. Reductions in digestibility may be due to the "carbohydrate effect" (Arroquy et al., 2005). According to Arroquy et al. (2005), the "carbohydrate effect" decreases digestion because ruminal microbes have a greater affinity for more readily digestible nutrients, and the decreased cellulose digestion is not entirely caused by a reduced pH. Wheat is more readily digestible than low-quality forages, which may also explain the lack of differences observed in the current experiment.

Performance

Average daily gain among treatments was not different ($P = 0.54$; Table 3), but G:F was less ($P < 0.01$) for steers consuming the SUPP. The decrease in the G:F is due to an increase in DMI without a concurrent increase in ADG. Horn et al. (1995) supplemented steers grazing wheat pasture with a 1.22 kg/d of ground corn, and reported a greater ADG for steers consuming the ground corn supplement compared to steers without supplementation. Hess et al. (1996) supplemented steam-flaked corn at 0.34% of BW while ruminally-cannulated steers grazed endophyte-free fescue and reported increased ADG of steers consuming the supplement compared to steers not supplemented. The wheat forage having similar energy content to SUPP may explain the lack of performance differences between treatments. The SUPP in the current experiment was 69.3% concentrate. Had SUPP been 100% concentrate, performance advantages may have been observed.

Gaseous Emissions and Energetic Losses

Whole-body calorimetry is the standard method for measuring gaseous emissions and energy metabolism of cattle. However, whole-body calorimeters cannot measure gaseous emissions of grazing animals. The GF system is portable, and more than one animal can utilize the system. Thus, GF can accompany animals in a pasture setting, and potentially account of environmental factors not accounted for in the controlled environment of whole-body calorimetry. With the use of respiratory quotients and the heat production equation of Brouwer (1965), one can estimate net energy.

Carbon dioxide excreted (kg/d) tended ($P = 0.08$; Table 4) to increase with SUPP. Methane emissions (g/d) were not influenced ($P = 0.43$) by treatment. However, CH_4 excreted per kg of DMI was decreased ($P = 0.02$) by 20.5% for steers consuming SUPP. The SUPP did not affect ($P = 0.11$) CH_4 emissions when expressed as g/kg of NDF intake. There were no differences ($P = 0.43$) in Mcal of CH_4 produced between treatments, but CH_4 as a percent of GE intake was decreased ($P = 0.02$) 21.6% when steers consumed the SUPP. Lastly, Mcal of heat production (**HP**) produced was not different ($P = 0.11$) among treatments, but HP as percent of GE intake was decreased 20.6% for steers consuming the SUPP.

Carbon dioxide suppression indicates increased rumen metabolism. Methane lost as a percent of GE intake in the current experiment concurs with reported CH_4 emissions (Johnson and Johnson, 1995). Methane lost as a percent of GE intake is predicted to be 6 to 8%, and the numbers observed from the GF system in the present experiment (CON: 8.21%; SUPP: 6.44%) aligns with those predictions. The reduction CH_4 as a percent of GE intake in the current trial between treatments may be attributed to the greater propionate production at the expense of acetate production (Hristov et al., 2013). The greater amount of propionate produced in the rumen causes a reduction in available hydrogen for CH_4 production. Olson et al. (1999) reported increased ruminal propionate production with starch supplemented at a minimum of 0.15% of BW. In the current experiment, without measuring ruminal VFA production, the most likely reason for the reduction in CH_4 as a percent of GE intake is due to the increased total DMI by steers consuming the SUPP. Johnson and Johnson (1995) described an increased level of intake decreases CH_4 by 1.6% with each level. Steers consuming the SUPP consumed a greater amount of GE, means CH_4 excreted (g/d) can be divided among a larger amount of GE intake. Thus, a smaller loss of CH_4 as a percentage of GE intake is observed.

IMPLICATIONS

Under the conditions of this experiment corn supplementation to cattle grazing wheat pasture altered CH_4 emissions, most likely from increased DMI. Corn supplementation may be a potential opportunity to mitigate CH_4 emissions of livestock grazing forage and help relieve concerns over livestock air pollution.

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Table 3. Performance and nutrient digestibility of steers grazing wheat pasture and consuming a corn-based supplement

	Treatment[1]			
Item	CON	SUPP	SEM	*P*-value
Initial BW, kg	417	417	5.6	0.95
Final BW, kg	497	497	6.7	0.96
DMI, kg	14.4	18.1	0.75	<0.01
Forage intake, kg[2]	14.0	15.8	0.75	0.13
Forage intake, % of BW[2]	3.22	3.61	0.181	0.15
ADG, kg	1.4	1.3	0.07	0.54
G:F	0.098	0.073	0.0054	<0.01
App. total tract digestibility, %				
OM	84.9	84.6	0.47	0.71
NDF	82.5	83.1	0.84	0.63

[1]CON = 0.2kg of pelleted wheat middlings; SUPP = supplemented 0.5% BW of corn-based supplement plus 0.2 kg of pelleted wheat middlings.

[2]Determined using IVDMD of wheat and TiO_2 as a marker for fecal output.

Table 4. Gas emissions of steers grazing wheat and consuming a corn-based supplement

	Treatment[1]			
Item	CON	SUPP	SEM	*P*-value
No. of observations	12	11		
CO_2, kg/d[1]	9.8	10.5	0.30	0.08
CH_4, g/d[1]	334	351	15.0	0.43
CH_4, g/kg of DMI	24.4	19.4	1.48	0.02
CH_4, g/kg of NDF intake	63.7	53.8	0.42	0.11
CH_4 energy, Mcal/d	4.4	4.6	0.20	0.43
CH_4, % of GE intake	8.21	6.44	0.499	0.02
Heat production, Mcal/d[1]	25.3	27.2	0.81	0.11
% of GE intake	47.86	38.01	3.147	0.03

[1]CON = 0.2kg of pelleted wheat middlings; SUPP = supplemented 0.5% BW of corn-based supplement plus 0.2 kg of pelleted wheat middlings.

[2] Collected using a GreenFeed device (C-Lock, Inc.; Rapid City, SD)

[3] Calculated using the Brouwer (1965) equation, assuming an RQ of 1.05, and assuming urine energy is 1.4% of GEI.

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Mammalian hormones associated with stress impact microbial fermentation of rumen fluid in vitro[1]

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ABSTRACT: Mammalian stress hormones may negatively impact bacteria found in the digestive tract, which could be harmful to animals undergoing stress such as newly received cattle. This study evaluated the effects of epinephrine, norepinephrine, and cortisol on rumen microbial fermentation and gas production in vitro. Treatments included no stress hormone (CON), epinephrine (EPI), norepinephrine (NOR), cortisol (CORT), and a combination of EPI, NOR, and CORT (ALL). Catecholamine treatments were added to fermentation flasks at 1.125 ng/mL, and the cortisol treatment was added at 1.15 ng/mL of rumen fluid. At initiation of the study, rumen fluid was collected from two ruminally-cannulated cows, homogenized with McDougal's artificial saliva, and inoculated with one of 5 treatments for 5 consecutive periods. The rumen microbial fermentation products ammonia (NH_3) and volatile fatty acids (VFA) were sampled at h 0, 2, 4, 6, 8, and 12 during each of the first 4 periods to produce a total of 120 in vitro fermentation samples. The pH was also measured at each collection time. Gas production was measured during the final period at h 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 from 24 fermentation flasks in which the 5 treatments were present in 4 replicates allowing for 4 blanks. Neither pH, NH_3, nor total VFA concentration were different ($P \geq 0.40$) among treatments. Molar percentages of acetate and isovalerate in rumen fluid were lower ($P < 0.01$) for EPI and NOR than CON, CORT, and ALL. Conversely, molar percentages of butyrate in rumen fluid were greater ($P = 0.03$) for EPI and NOR than CON, and intermediate for CORT and ALL. A treatment × hour interaction ($P < 0.01$) was observed for gas production from 8 h of incubation to the culmination of this experiment, indicating that microbial fermentation is altered by treatments.

Key words: Catecholamine, Cortisol, Fermentation, In Vitro, Stress

INTRODUCTION

Physical and psychological stress on animals has been shown to increase circulating levels of catecholamines (epinephrine and norepinephrine; Houpt et al., 1988), as well as the steroid hormone cortisol (Yates et al., 2010). Many sectors of the beef production industry are negatively impacted by the detrimental effects of stress on cattle. Most notably, newly weaned calves from cow-calf operations often experience high levels of stress during transport and assimilation to new environments, such as feedlots (Galyean et al., 1981).

At onset of stress, activation of the hypothalamo-pituitary-adrenocortical axis (HPA) and sympatho-adrenal medullary axis initiates secretion of catecholamines and glucocorticoids (Minton, 1994). Increased concentrations of cortisol, epinephrine, and norepinephrine are detectable in both plasma and saliva of sheep and cattle (Houpt et al., 1988; Minton, 1994; Yates et al., 2010). Walker and Drouillard (2012) and Freestone and Lyte (2010) have discussed the possibility of a direct connection between increased concentrations of circulating stress hormones and a decrease in immunity because of their effects on the animal as well as interactions with many bacterial species. For example, Freestone and Lyte (2010) observed that catecholamines increase the growth of gram-negative bacteria, importantly the pathogenic microorganisms (*E. coli* O157:H7, *Salmonella enterica*, and *Yersinia enterocolitica*) that reside in the digestive tract of cattle.

We hypothesized that salivary concentrations of cortisol, epinephrine, and(or) norepinephrine could alter microbial activity in the rumen. Therefore, the objective of this study was to evaluate the effects of these key mammalian hormones on rumen microbial fermentation and gas production in an in vitro experiment.

MATERIALS AND METHODS

Fermentation Conditions

Use of ruminally-cannulated cows for collection of rumen fluid was approved by the New Mexico State University Institutional Animal Care and Use Committee. At initiation of each fermentation period, approximately 1 L of rumen fluid was collected from each of 2 ruminally-cannulated cows via a suction strainer (Precision Machine Co, Lincoln, NE). Equal parts rumen fluid from each cow were combined in a pre-heated (± 39°C) thermos to obtain a total volume of 2 L, and immediately transported to the laboratory. The rumen fluid was then mixed with an equal volume of prepared McDougal's buffer (May and Galyean, 1996) that had been pre-heated (± 39°C) and flushed with CO_2 to obtain a pH between 6.8 and 7.0. McDougal's-buffered rumen fluid (100 mL) was decanted into 250-mL Erlenmeyer flasks each containing 1 g of ground substrate (Table 1) and 1 of 5 randomly assigned treatments (see below). Flasks were then flushed with CO_2, fitted with rubber stoppers containing one-way gas release valves, and

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incubated at 39°C in a LAB-LINE Orbit ENVIRON-Shaker (LAB-LINE INSTRUMENTS, Inc., Designers and manufacturers, Melrose Park, IL).

Experimental Design

Two experiments were conducted, one for determination of gas production and the other for measurements of rumen fluid fermentation end-products and pH. The gas production experiment was a completely randomized design, where 20 Erlenmeyer flasks containing McDougal's-buffered rumen fluid, ground substrate, and treatments, and 4 'blank' Erlenmeyer flasks (excluded rumen fluid and treatments) were incubated continuously for 12 h. The rumen fluid fermentation end-products experiment was a randomized complete block design, where 120 Erlenmeyer flasks containing McDougal's-buffered rumen fluid, ground substrate, and treatments were incubated for 0, 2, 4, 6, 8, or 12 h. Because of a limited amount of space in the incubator, the experiment was conducted over 4 incubation periods (days) and blocked by day of incubation. Erlenmeyer flasks assigned to 0 h of incubation were not placed into the incubator. Treatments were randomly assigned to Erlenmeyer flasks within each block.

Treatments

Treatments were: 1) no stress hormone (**CON**), 2) cortisol (**CORT**), 3) epinephrine (**EPI**), 4) norepinephrine (**NOR**), and 5) a combination of CORT, EPI, and NOR (**ALL**). Using a calibrated pipette, 100 µL pre-prepared stress hormone solutions were added directly to each randomly assigned Erlenmeyer flask to supply 1.12 ng of EPI or NOR and 1.15 ng of CORT per mL of McDougal's-buffered rumen fluid. To prepare the cortisol solution, 23 mg of hydrocortisone powder (Sigma Aldrich, St. Louis, MO) was mixed with 20 mL of ethyl alcohol (200 proof; Pharmco-Aaper, Brookfield, CT) 3 d before beginning the incubations. To prepare the epinephrine and norepinephrine solutions, 5.6 mg of each hormone (Sigma Aldrich, St. Louis, MO) were mixed with 5 mL of deionized water. To avoid oxidation, the epinephrine and norepinephrine solutions were made 1 h before the beginning of each incubation period. Erlenmeyer flasks that were assigned to CON received 100 µL of ethyl alcohol only.

Collections

For the gas production experiment, volume of total gas was measured using water displacement as described by Alford et al. (2014). Measurements of gas were recorded at 15, 30, 60, 90, and 120 min., and then at 1 h intervals for 12 h.

For the rumen fluid fermentation end-products experiment, Erlenmeyer flasks were removed from the incubator after 0 (not incubated), 2, 4, 6, 8, or 12 h of incubation, and the pH of the McDougal's-buffered rumen fluid was immediately measured using a portable pH meter (Mettler-Toledo AG, CH-8603 Schwerzenbach, Switzerland). Then, a sample (10 mL) of McDougal's-buffered rumen fluid was transferred to a 15 mL conical tube containing 2 mL of 25% (w/v) meta-phosphoric acid (Sigma Aldrich, St. Louis, MO) solution, immediately placed on ice to arrest fermentation, and frozen at -20°C for later analysis of VFA. Similarly, 10 mL of McDougal's-buffered rumen fluid was collected into a 15 mL conical tube containing 2 mL of 5% hydrochloric acid (HCl), placed on ice, and frozen at -20°C for analysis of NH_3. Samples were analyzed for VFA concentrations using gas chromatography (Agilent 7890A, Agilent Technologies, Santa Clara, CA) according to the methods of May and Galyean (1996). Rumen fluid NH_3 concentrations were analyzed using the methods of Broderick and Kang (1980) adapted to a microtiter plate (BioTek Instruments, Winooski, VT) and read at an absorbance of 630 nm.

Statistical Analysis

Data was analyzed as repeated measures using the MIXED procedure of SAS (SAS Version 9.3 Inst. Inc., Cary, NC). The gas production experiment was analyzed as a completely randomized design, and the rumen fluid fermentation end-products experiment was analyzed as a randomized complete block design. Erlenmeyer flask was the experimental unit, and the statistical model included the effects of treatment, hour, and the treatment × hour interaction. Differences of $P < 0.05$ were considered significant.

Table 1. Composition of ground substrate

Item	% of DM
Ingredient	
Grain, Corn cracked	41.0
Hulls, Soybean	20.0
Hay, Sudan	15.0
Dried distiller's grains	15.0
Molasses	8.0
Urea	0.50
Limestone	0.30
Salt	0.20
Nutrient[1]	
NDF	28.02
ADF	18.65
CP	15.61
Ca	0.41
P	0.43

[1]Analyzed by SDK Laboratories, Hutchinson, KS

RESULTS

A treatment × hour interaction ($P < 0.01$) was observed for gas production (Fig. 1); gas production was not different among treatments before 7 h of incubation, whereas from 7 to 12 h gas production tended to be lower or was lower for EPI and ALL than CON and CORT, and intermediate for NOR.

No treatment × hour interactions ($P \geq 0.59$) occurred for pH, NH_3, total VFA, molar concentrations of individual VFA, and acetate:propionate ratio (data not shown). Similarly, pH, NH_3, and total VFA were not different ($P \geq 0.40$) among treatments (Table 2). Molar percentages of acetate and isovalerate in rumen fluid were lower ($P < 0.01$)

for EPI and NOR than CON, CORT, and ALL. Molar percentages of butyrate in rumen fluid were greater (P = 0.03) for EPI and NOR than CON, and intermediate for CORT and ALL. Acetate to propionate ratios, and molar percentages of propionate, isobutyrate, and valerate were not different ($P \geq 0.45$) among treatments.

DISCUSSION

Among various cattle breeds, typical rumen volume is between 60 to 100 L and approximately 20.0 L of saliva is produced per day (Church, 1993). In the current study, epinephrine and norepinephrine were added to individual flasks containing McDougal's-buffered rumen fluid at concentrations of 1.12 ng/mL, whereas cortisol was added to flasks at 1.15 ng/mL. These values were designed to provide total rumen concentrations of stress hormones similar to those that would be achieved by typical salivary flow of ruminants containing 9 ng/mL of each hormone for a 12 h period (assuming a rumen volume of 80 L and a flow rate of 10 L per 12 h). However, because the mammalian gut is innervated with sympathetic nerve terminals that are dispersed throughout the enteric nervous system (ENS), it is likely that greater concentrations of stress hormones may be present in the digestive tract than those supplied in the current study (Freestone et al., 2007). Greater concentrations, specifically those of the catecholamines, could potentially amplify the results seen on fermentation parameters measured in this experiment.

Lower gas production was observed for EPI and ALL compared with CON suggesting that epinephrine had the most negative effect on rumen microbial fermentation. There was also a tendency for gas production to be lower with the NOR treatment compared with CON. This is interesting because more norepinephrine is present than epinephrine in mammalian species under normal conditions and are up-regulated with onset of acute stress (Lyte, 2004) leading researchers of this study to believe that the NOR treatment would elicit a stronger response than EPI. Nevertheless, it was apparent that treatments containing norepinephrine, epinephrine, or both elicited a larger response from bacteria in terms of gas production than those that contained cortisol or no stress hormones. Catecholamines have been shown to upregulate growth of certain species of bacteria in culture (O'Donnell et al., 2006), which could explain greater gas production of rumen bacteria exposed to catecholamines. However, microbial populations were not sampled or cultured in the present study, so the exact mechanism behind increased gas production cannot be determined.

Although a difference in gas production was observed, total VFA in rumen fluid was not different among treatments. Similarly, Walker et al. (2010) observed greater gas production but no differences in total VFA associated with the addition of the beta-agonist, ractopamine hydrochloride to fermentation flasks. Beta-agonists are structurally similar to the naturally occurring catecholamines and therefore may have produced a similar response in vitro to the epinephrine and norepinephrine treatments used in the current study (Walker and Drouillard, 2012). Despite no difference in total VFA, the molar percentage of acetate decreased for flasks that received norepinephrine and epinephrine. Interestingly, acetate concentrations were not affected by the treatment containing all of the stress hormones, which suggests that different species of bacteria may respond differently to exposure to stress hormones (Walker and Drouillard, 2012).

IMPLICATIONS

These results imply that gas and volatile fatty acid production of rumen microbes are altered by moderate levels of stress hormones. In particular, exposure to high concentrations of catecholamines may influence bacteria efficiency in the rumen, whereas exposure to cortisol had little effect in the present study. Additional research needs to be completed to quantify how these observations relate to specific bacterial species and overall mammalian immunity.

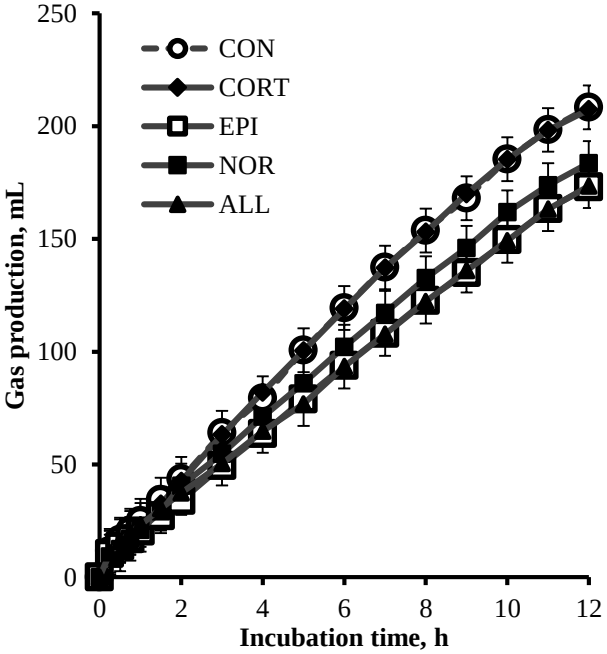

Figure 1. Gas production from McDougal's-buffed rumen fluid treated with no stress hormones (CON), epinephrine (EPI), norepinephrine (NOR), cortisol (CORT), and a combination of EPI, NOR, & CORT. Each hormone was supplied at ±1.13ng/mL of McDougal's-buffed rumen fluid. Effects were: treatment (P = 0.42), hour (P < 0.01), treatment × hour interaction (P < 0.01).

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Table 2. Effects of stress hormones on fermentation characteristics

	Treatments[1]						
	CON	CORT	EPI	NOR	ALL	SEM	*P*-value
pH	6.60	6.77	6.66	6.78	6.76	0.12	0.71
NH_3, m*M*	4.17	4.26	4.35	4.19	4.31	0.43	0.96
Total VFA, m*M*	108	106	106	103	107	4.52	0.40
VFA, mol/100 mol							
Acetate	66.6[a]	66.7[a]	65.9[b]	65.7[b]	67.0[a]	0.38	< 0.01
Propionate	14.8	14.8	15.0	14.8	13.8	0.80	0.49
Butyrate	14.2[c]	14.4[bc]	14.6[ab]	14.7[a]	14.5[abc]	0.50	0.03
Isobutyrate	1.81	1.57	2.10	2.33	2.12	0.48	0.54
Valerate	0.914	0.916	0.913	0.907	0.924	0.06	0.98
Isovalerate	1.66[a]	1.68[a]	1.53[b]	1.53[b]	1.66[a]	0.12	< 0.01
Acetate:propionate	4.64	4.66	4.54	4.60	4.99	0.27	0.45

[1]Treatments were no stress hormones (CON), epinephrine (EPI), norepinephrine (NOR), cortisol (CORT), and a combination of EPI, NOR, & CORT (ALL); each hormone was supplied at ±1.13ng/mL of McDougal's-buffed rumen fluid.
[a,b,c]Means in rows with different superscript letters differ ($P < 0.05$).

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Effects of rumen protected arginine supplementation to cows during early or late gestation on progeny glucose tolerance[1]

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ABSTRACT: Our hypothesis was calves gestated by dams supplemented rumen protected arginine during early or late gestation would have improved glucose tolerance. In order to test this hypothesis, a two yr study was conducted. Dams were randomly assigned to one of three treatments; 1) grazing native range plus dried distillers grain (Control), or grazing native range plus dried distillers grain and Arg fed to provide 180 mg L-Arg/kg BW either during 2) early gestation (EARG) or 3) late gestation (LARG). In yr 1, 16 yearling calves (heifers n = 8, steers n = 8) and in yr 2, 24 (heifers n = 10, steers n = 14) yearling calves underwent a glucose tolerance test (GTT). On the days of the GTT, cattle were fed at 0600 h and indwelling jugular catheters were inserted at 0700 h. A 50% dextrose solution was injected at 0.5 mL/kg BW via the jugular catheter and subsequent 6 mL blood samples were collected at -5, -2, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, and 120 min relative to the dextrose infusion. Glucose half-lives were estimated by regressing the logarithmically transformed glucose concentrations over time and area under the curve was determined using the trapezoidal summation method. Glucose area under the curve (AUC) did not differ (P = 0.13) between treatment groups; however, overall glucose concentration (conc.) tended (P = 0.06) to be lower for calves of arginine supplemented dams when compared with non-supplemented dams. There were no differences between treatment groups in reference to insulin AUC (P = 0.57), insulin half-life (P = 0.85), or overall insulin concentration (P = 0.47). In conclusion, rumen protected arginine supplementation to cows during varying times in gestation tends to affect overall glucose concentration in progeny during a glucose tolerance test; however, does not affect glucose or insulin AUC, half-life, or overall insulin concentration.

Key words: arginine, glucose AUC, glucose half-life

INTRODUCTION

Poor maternal nutrition can result in increased embryonic mortality rates and potentially generate offspring unable to perform at the level of their non-nutrient restricted counterparts (Barker, 1997; Larson et al., 2009; Funston et al., 2010 a,b). Nutrient restriction can negatively impact size and functionality of the placenta, thereby affecting subsequent blood supply, nutrient and oxygen availability and metabolic efficiency for the conceptus (Vonnahme et al., 2007; Wu et al., 2006). Recent research has indicated that poor placental production of nitric oxide (vasodilatory and angiogenic factor) and polyamines (crucial in DNA and protein synthesis) can contribute to poor fetal development during dam nutrient restriction (Wu et al., 2006). While it is understood that poor maternal nutrition can impact fetal development, the use of supranutritional levels of specific nutrients to enhance development are not well defined.

Arginine, a precursor for the production of nitric oxide and polyamines, may enhance maternal fetal blood exchange through increased placental vascularization. By improving maternal-fetal blood flow, gas, nutrient and waste exchange will also be improved, thus providing maximal nutrients for organogenesis (i.e. pancreatic development). An in vitro study conducted by Rhoten (1980) reported fetal rat pancreata were perinfused with low or high concentrations of glucose in the presence or absence of arginine and leucine. This study demonstrated that fetal rat pancreas development was enhanced when arginine was supplied during gestation (Rhoten, 1980). The hypothesis of this study is calves born to cows supplemented with arginine during gestation will have improved glucose utilization. The main objective is to investigate differences in glucose metabolism of progeny born to dams supplemented arginine during early or late gestation when compared with those born to dams receiving no arginine supplementation.

MATERIALS AND METHODS

All animals and procedures were handled in accordance with the New Mexico State University Institutional Animal Care and Use Committee.

Cow Management and Treatments

A two year study utilized mature Angus × Hereford cows at the Coronal Range and Livestock Research Center (CRLRC) in Corona, NM. All cows were confirmed pregnant to AI 30 d post insemination via blood analysis for pregnancy specific protein B (BioTracking Inc., Moscow, ID). Cows were utilized in a completely randomized design and randomly assigned one of three dietary treatments: 1) grazing native range (average across yr of 95.4% DM, 5.9% CP, 74.18% NDF, DM basis) plus dried distillers grain (**CON**), or grazing native range plus dried distillers grain and rumen protected arginine (55% rumen protection; Miller, 2012) fed to provide 180 mg L-Arg/kg BW either during 2) early gestation (**EARG**; starting on d 55 of gestation) or 3) late gestation (**LARG**; starting on d 210 of gestation). In yr

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1 (2012) 16 cows (CON n = 6, EARG n = 6, LARG n = 4) were utilized and yr 2 (2013) had 27 cows (Control n = 7, EARG n = 10, LARG n = 10). All cattle were gathered and supplemented individually 3 times per wk for 30 d and the basal diet (DDGs and native range land) was calculated to provide 155% of their arginine requirement (NRC, 2000). A level of 180 mg/kg BW of arginine has been shown to improve vascular hemodynamics in steers (Meyer et al., 2011) and sheep (Saevre et al., 2011). Supplementation windows selected correspond to key points in fetal development that include maximal placental growth, organ differentiation, vascularization and organ growth (Funston et al., 2010a).

Progeny Management

Cows were managed as a single group throughout the experiments at the CRLRC. Following weaning calves were preconditioned for 60 d at the ranch after which calves were transported to New Mexico State University campus in Las Cruces, NM for adaptation to a Calan Gate feeding system (American Calan, Northwood, NH) in order to undergo a subsequent backgrounding trial (yr 1: 56 d and yr 2: 42 d trial) where they were offered ad libitum access to a grower diet (Yr 1 – 19.31% CP, 0.43 NEm, 0.25 NEg; Yr 2 – 10.5% CP, 2.02 NEm, 1.19 NEg).

Glucose Tolerance Test

Following completion of the feed trial, all calves were subjected to a glucose tolerance test (GTT). In each yr, the GTT was conducted over the course of 2 days where steers were tested the first day and heifers were tested the next. On the day of the test, calves were fed at 0600 and catheter placement began at 0700. Following the collection of three baseline samples (time points -5, -3, 0), a 50% dextrose solution was infused at 0.5 mL/kg of BW via the jugular catheter. Blood samples were then collected at time points 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, and 120 min relative to infusion. At each sampling, 7 mL blood was drawn into a sterile syringe that was then dispensed into tubes containing 15 mg of NaF and 12 mg K oxaloate (BD Vacutainer, Franklin Lakes, NJ). Samples were then placed on ice for 30 min and centrifuged at 1,000 x *g* for 20 min at 4°C and plasma was removed and stored at -20°C. Following the collection of the blood sample, 10 mL of saline was inserted into the catheter to clear the catheter until the next blood draw.

Sample analysis

Glucose was analyzed in triplicate using a colorimetric procedure. All plasma samples were diluted by 50% with DI water. Utilizing a 90 well plate, two microliters of standard solutions, controls (BSA), and diluted samples were pipetted into their respective wells. Then 300 microliters of glucose hexokinase was added to each well. The plate was then run using a Synergy H1 Hybrid Plate Reader (BioTek) (Long et al., 2010b). Intra- and inter-assay coefficients of variation averaged 3.84%. Plasma insulin concentrations were determined using a double antibody RIA as described by Sanson and Hallford (1984). Inter-assay CV averaged 8.7% and intra-assay CV average 6.4%.

Calculations and Statistical Analysis

Data were analyzed as a completely randomized design using PROC MIXED (SAS Inst. Inc., Cary, NC). The model included treatment, year, sex and treatment × sex and treatment × year interactions. Glucose half-lives were estimated by regressing the logarithmically transformed glucose concentrations over time. Area under the curve was determined using the trapezoidal summation method as described by Long et al. (2010b). Means were separated using LSMEANS. No interactions were significant ($P \geq 0.22$). A P-value of ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

All glucose tolerance test data is presented in Table 1 and Figure 1 and 2. Prior to glucose infusion, calves born to cows fed arginine during EARG had lower initial blood glucose level ($P = 0.01$) when compared with LARG and CON (Table 1). This is interesting due to the fact that all calves consumed the same diet (39.7% DM, 10.5% CP, 2.02 Mcal/kg NEm, 1.19 Mcal/kg NEg) and dry matter intake did not differ ($P \geq 0.25$) between treatment groups prior to the glucose challenge. Overall glucose concentrations did not differ between groups ($P = 0.14$); however, numerically, EARG calves had lower overall glucose concentration. Calves from arginine supplemented dams (EARG and LARG) tended to have lower overall blood glucose concentrations ($P = 0.06$) compared to CON. In yr 1, glucose area under the curve (AUC) tended to be lower ($P = 0.06$) for the EARG group and those calves in the arginine groups (EARG and LARG) tended to have a smaller glucose AUC ($P = 0.11$); however, no differences were found in glucose AUC in yr 2 calves ($P = 0.55$). When yr 1 and 2 data were combined, no differences for glucose AUC ($P = 0.13$) were observed between treatments, with only numerical differences being observed, with EARG having numerically lower glucose AUC. Further, glucose half-life did not differ between treatment groups ($P = 0.95$). Insulin AUC, half-life, and overall concentration did not differ ($P = 0.57$; $P = 0.85$; $P = 0.78$, respectively). Insulin AUC was different ($P = 0.0008$) between yr 1 and yr 2 calves; however, no other sex, year, treatment x sex or treatment x year interactions differed ($P \geq 0.43$ and $P \geq 0.45$, respectively) for any other measurements.

Due to our current small sample size, only numerical differences were observed, nevertheless our results do agree with previous data (Ford et al., 2007; Long et al., 2010a; Zhang, 2010) where it was found that maternal nutrition can affect offspring's ability to metabolize glucose. Ford et al., (2007) found that lambs born to nutrient restricted ewes exhibited a significantly larger glucose AUC ($P < 0.05$) and insulin AUC ($P < 0.001$) as determined during a glucose tolerance test when compared to lambs born to non-restricted ewes at d 63 of age. Furthermore, lambs of nutrient restricted dams displayed a larger glucose AUC ($P < 0.01$), but a smaller insulin AUC ($P = 0.05$; Ford et al., 2007). Our original overarching hypothesis is that arginine supplementation during early gestation will affect the development of key organs such as the pancreas during fetal development. Numerical differences and near tendencies reported in the present study suggest calves born to arginine supplemented dams may have a increased ability to absorb glucose from the blood into the tissues. This may indicate that arginine supplementation, especially during early gestation, is beneficial to pancreatic development and maturation during fetal development. However, other mechanisms involving the insulin dependent glucose

transporter, GLUT 4, may play a role. For example, Long et al. (2010c) found that steers born to nutrient restricted dams displayed a decreased amount of GLUT 4 in the peri-renal adipose tissue (Gardner et al., 2005; Long et al., 2010c); however, contradicting results were found in a separate study where calves born to cows on a low-plane of nutrition had an increased time to plasma glucose clearance (Long et al., 2010b). The results obtained thus far indicate arginine supplementation during gestation may influence offspring glucose metabolism; however, the mechanism behind this observation has yet to be determined. Therefore, current work is being conducted to examine pancreatic tissues from calves to determine differences in Islet of Langerhans numbers, insulin secretion by pancreatic beta cells, and pancreatic insulin.

Table 1. The effects of maternal Arg supplementation during early or late gestation on glucose and insulin area under the curve (AUC) and glucose half-life after glucose tolerance test

	Treatments[1]				
Item	CON	EARG	LARG	SE	*P*-value
n	13	15	12		
Glucose					
AUC[2]	518.4	499.9	520.4	8.3	0.13
Half - life, min[3]	102.6	99.7	104.5	11.0	0.94
Initial conc., mg/dL	68.4[a]	55.8[b]	68.9[a]	3.8	0.01
Overall conc., mg/dL	108.6	99.6	102.5	3.4	0.14
Insulin					
AUC[2]	742.4	727.9	725.8	23.3	0.85
Half -life, min[3]	90.1	76.8	96.1	16.9	0.61
Overall conc., ng/dL	6.2	1.8	7.9	4.1	0.47

[a-b]Means with different superscripts differ $P \leq 0.05$.

[1] Dams grazed native range and were provided dried distillers grain (CON), or grazing native range plus dried distillers grain and rumen protected arginine (55% rumen protection) fed to provide 180 mg L-Arg/kg BW either during early gestation (EARG; starting on d 55 of gestation) or late gestation (LARG; starting on d 210 of gestation).

[2]Area under the curve was determined using the trapezoidal summation method.

[3]Half-life was calculated as the time required for a 50% decrease in peak plasma glucose and insulin concentration.

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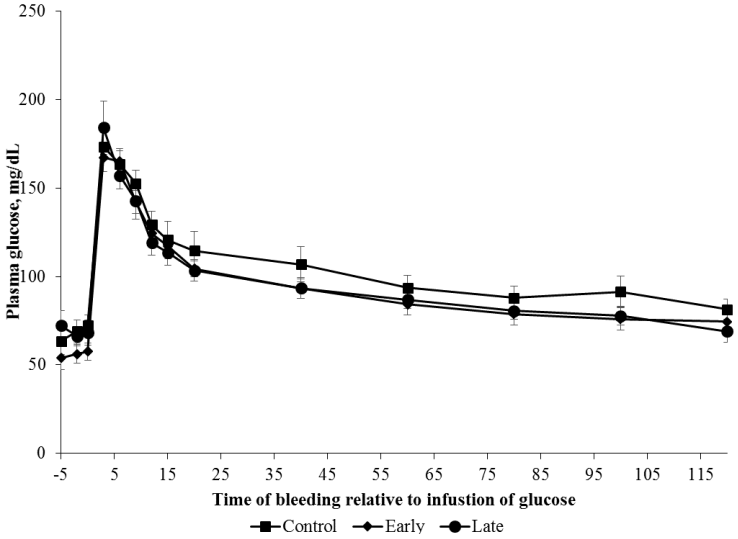

Figure 1. The effects of maternal Arg supplementation during early or late gestation on progeny plasma glucose concentrations after performing a glucose tolerance test. Effects for the entire bleeding period included: treatment ($P = 0.28$), time ($P < 0.0001$), treatment × time ($P = 0.58$), sex ($P = 0.64$), treatment x sex ($P = 0.11$), and year ($P = 0.04$).

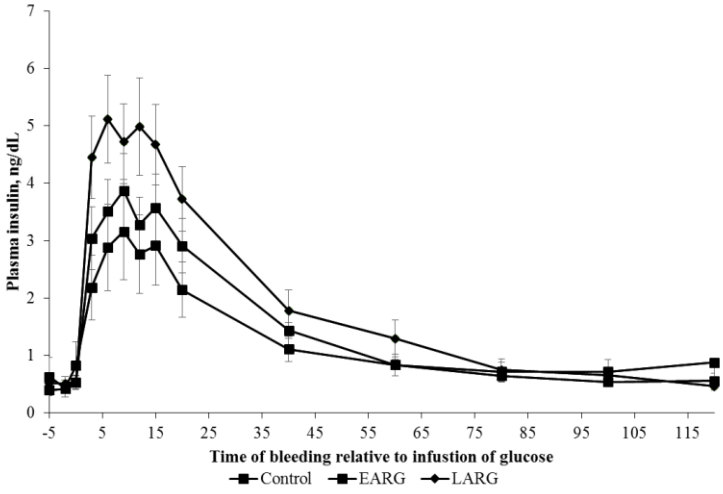

Figure 2. The effects of maternal Arg supplementation during early or late gestation on progeny plasma insulin concentrations after performing a glucose tolerance test. Effects for the entire bleeding period included: treatment ($P = 0.47$), time ($P = 0.02$), treatment x time ($P = 0.98$), sex ($P = 0.15$), treatment x sex ($P = 0.57$), year ($P = 0.12$) and treatment x year ($P =$

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Effects of administering Ralgro to Holstein calves during the hutch period on growth performance

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ABSTRACT : We hypothesized that early administration of a Ralgro implant to one day old Holstein calves will improve growth performance. One thousand two hundred and forty-eight one day old Holstein steer calves (initial BW 41.2 ± 0.2 kg) were utilized in a completely randomized block designed experiment with truck load serving as the block (6 loads). On d 0, calves were individually weighed, tagged with an electronic identification tag, and vaccinated with Vision CD-T with Spur during initial processing. Calves were randomly assigned within block to receive one of two treatments: 1) growth implant containing 36 mg Zeranol (n = 584) or no growth implant (n = 598). Calves were individually housed in wood hutches and provided ad libitum access to grain starter (17.2% CP, 4% fat and NEm 0.49 Mcal/kg and NEg 0.34 Mcal/kg) and water. In addition, calves were offered two, 1.9 L bottles of milk replacer two times daily (25.5 % CP and 22.5% fat, DM basis). Implanted calves had greater DMI ($P < 0.01$) compared to non-implanted calves over the 92-d period. Likewise, ADG was greater ($P < 0.01$) when calves were implanted at 1 day of age versus non-implanted calves. However, due to the increase in DMI and ADG for calves receiving implants, G:F tended to differ ($P = 0.08$) between treatments. Overall, implanting calves with 36 mg of Zeranol did not appear to have any adverse effects on intake or feed efficiency in one day old calves during the hutch phase and improved ADG by 6%.

Key words: Calf, Growth, Hutch, Implants, Zeranol

INTRODUCTION

The growth of the dairy industry has caused an increase in the number of dairy beef calves finished each year. Therefore, dairy beef producers are seeking ways to increase growth and carcass quality of calves to decrease time on feed prior to entry into the feedlot. One effective way to increase growth in calves is by administration of growth implants. This is common practice for most feedlots in older calves. However, very little work has been done to investigate the impact of growth implants on newborn Holstein calves (1 to 2 days of age). Donovan et al. (1983) observed that rate of gain was increased by 10.7% when suckling Holsteins were implanted with 36 mg zeranol at birth, and a further 3% increase in growth occurred if the calves were reimplanted 84 days later. Finding an effective tool for improving profit in dairy calf ranches has potentiated the idea of implementing implants in particular Holstein calves at a younger age and through the finishing period. What is not known is if implanting dairy beef steers at such a young age will have a positive impact on growth. Therefore, we hypothesized that early administration of a growth implant on one day old Holstein calves will increase daily gain and feed efficiency. The objectives were to determine the effect of 36 mg of Zeranol administered to one day old Holstein calves on DMI, ADG, and G:F during a 92-d hutch period.

MATERIALS AND METHODS

All procedures were conducted in accordance to the rules of the New Mexico State University Institutional Animal Care and Use Committee.

Experimental design and treatments

One thousand two hundred and forty-eight Holstein steer calves (initial BW 41.2 ± 0.2 kg) 1 to 2 d of age were purchased and transported to Reynolds Creek Calf Ranch in Melba, Idaho. Calves arrived at the calf ranch between March 18, 2015 through April 22, 2015 (6 loads). All steers had access to water upon arrival and throughout the trial, and all cattle were processed within 24 hours of arrival. On d 0, calves were individually weighed, tagged with an electronic identification tag, given a visual ear tag, and vaccinated with Vision CD-T with Spur. The experiment was a completely randomized block design, with load serving as the block. Calves were randomly assigned within block to receive one of two treatments (n = 624): 1) growth implant (36 mg Zeranol) or no growth implant. Calves were individually housed in plywood hutches and provided ad libitum access to grain starter (17.2% CP, 4% fat and NEm 0.49 Mcal/kg and NEg 0.34 Mcal/kg) and were offered two, 1.9 L bottles of milk replacer two times daily (25.5 % CP and 22.5% fat, DM basis) initially and were gradually weaned off of milk over the course of the first 70 d and as grain intake increased. The amount of feed offered was adjusted using daily visual estimates of unconsumed feed amounts remaining in the bunk. Calves were checked for abscesses that may have occurred due to the implant procedure. Throughout the trial, the on-site veterinarian monitored health and by d 92, 66 calves were removed from the data set due to morbidity or mortality (non-implant = 26 calves and implant = 40 calves).

Statistical Analysis

Data were analyzed as a completely randomized block design with load serving as the block. Experimental unit was calf (n = 598). All data were analyzed using the PROC MIXED procedure of SAS (9.3; SAS Inst. Inc., Cary, NY) with treatment and block as the fixed effects.

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Treatments differed at a $P < 0.05$ and tended to differ at $P < 0.10$.

RESULTS AND DISCUSSION

Initial body weight did not differ ($P = 0.20$) among treatments (Table 1). Implanted calves had greater DMI ($P < 0.01$) compared to non-implanted calves over the 92-d period. Calf d-92 BW was greater ($P < 0.01$) for implanted calves compared to non-implanted. Donovan et al. (1983) implanted Holstein bull calves with 36 mg zeranol within the first week of entry into hutch phase and then reimplanted at 90 d with same implant and found that implanted calves gained 2.28 kg more than non-implanted calves, which is similar to the 3 kg improvement in overall BW gain for implanted calves in the current study. Implanting calves increased ($P < 0.01$) ADG. Blome et al. (2003) reported an ADG 0.62 kg/d in calves receiving 25.8% CP milk replacer, which is similar to the ADG of implanted calves (0.624 kg/d) reported herein. Average daily gain is an economic driver in the first 90 days on feed for calves at the calf ranch due to the high feed cost associated with milk replacer. Stimulating intakes is also advantageous in allowing calves to convert from a milk diet to a grain diet more quickly thus allowing for a lower cost of production. Overall feed efficiency, expressed as G:F, tended to differ ($P = 0.08$) across treatments. These findings are similar to those of Smith et al. (1999) and Hermesmeyer et al. (2000), who noted that implanted cattle had improved feed efficiency relative to non-implanted cattle.

Increases in DMI and ADG are common responses observed in the literature when ruminants are implanted (NRC, 1994). The increase in DMI was largely due to increased grain consumption as milk was limit fed. Cheatham et al. (2008) reported no differences in G:F in implanted feedlot steers compared to non-implanted steers. Likewise, Egan et al. (1993) also reported no difference in G:F when veal calves were implanted with increasing levels of Zeranol at 3 to 7 d of age. Conversely, Smith et al. (1999) implanted male Holstein veal calves at 3 to 7 d of age and reported increases in G:F during the first 42 d of the experiment. In most experiments investigating the efficacy of implants, a positive response in ADG and intake are observed, however, it appears that when G:F does not differ, the magnitude of increase in dietary intake is such that G:F improvements are not detected.

In conclusion, the dairy beef industry is continually looking for ways to decrease costs of production. Implanting calves at a very young age can improve growth performance and ultimately decrease time to target weight for entry into the feedlot.

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Table 1. Effects of implanting newborn Holstein calves with 36 mg of Zeranol on ADG during a 92-d hutch period

	Treatment			
Item	No Implant	Implant	SE	*P*-value
n	598	584		
BW, kg				
d 0	41.4	41.1	0.2	0.20
d 92	95.0	97.5	0.5	< 0.01
DMI, kg/d	1.34	1.38	0.01	< 0.01
ADG, kg/d	0.582	0.614	0.005	< 0.01
G:F kg gain/kg feed				
	0.435	0.443	0.003	0.08

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Effects of protein concentration and degradability on performance and carcass characteristics of finishing heifers receiving 0 or 400 mg ractopamine hydrochloride[1]

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ABSTRACT: This study evaluated if excess protein decreases performance and carcass quality of finishing cattle fed diets with or without ractopamine hydrochloride (**RH**). Heifers were assigned to 48 pens in a randomized complete block design and pens of cattle were randomly assigned to 3 protein and 2 RH (0 vs 400 mg/day) treatments. Protein treatments were steam-flaked corn-based diets containing 13.9% CP, 8.8% RDP, and 5.0% RUP **(CON**), 20.9% CP, 13.4% RDP, 6.1% RUP **(High RDP**), or 20.9% CP, 9.1% RDP, 10.4% RUP (**High RUP**). Cattle were weighed at initiation of RH and at shipping. No RH × CP interactions ($P \geq 0.11$) occurred for performance or carcass traits. Excess CP did not affect ($P \geq 0.12$) final BW or ADG. Carcass-adjusted final BW and ADG tended to be greater ($P = 0.06$) for cattle receiving High RDP than High RUP and CON. Water intake, DMI, G:F, and carcass-adjusted G:F were not different ($P \geq 0.12$) among CP treatments. Hot carcass weight tended to be greater ($P = 0.06$) for cattle receiving High RDP than High RUP and CON. Dressing percentage was lower ($P < 0.01$) for cattle fed High RUP than High RDP and CON. Marbling score, 12th rib fat depth, LM area, and yield grade were not different ($P \geq 0.16$) among CP treatments. Heifers receiving High RUP tended to have lower ($P = 0.10$) KPH than CON. Percentage choice tended to be greater ($P = 0.09$) for heifers receiving High RDP vs. High RUP. Water and DMI were not different ($P \geq 0.36$) for RH vs. no RH. Cattle receiving RH had greater ($P < 0.01$) final BW, ADG, carcass- adjusted final BW, and carcass-adjusted ADG, and lower ($P < 0.01$) G:F and carcass-adjusted G:F compared with no RH. Hot carcass weights were greater ($P < 0.01$) and dressing percentage tended to be greater ($P = 0.09$) for cattle receiving RH, while marbling score was not affected ($P = 0.11$) by RH. Twelfth-rib fat depth tended to be lower ($P = 0.08$), and KPH was lower ($P = 0.02$) for RH vs. no RH. The LM area was greater ($P = 0.03$) for cattle receiving RH vs. no RH. Excess CP does not negatively impact performance or carcass traits of finishing cattle, and no interactions between CP and RH suggest that CP requirements are not affected by RH.

Key words: cattle, degradability, protein, ractopamine hydrochloride

INTRODUCTION

Increased availability of byproducts from the corn milling industry has altered the ingredient and nutrient composition of finishing cattle diets in the past 20 years. In particular, high amounts of byproducts in finishing cattle diets results in an excess supply of dietary protein. Previous research showed little or no improvements in cattle performance when CP concentrations of finishing diets exceeded 13% (Galyean et al., 1996; Gleghorn et al., 2004). However, few of these initial studies examined the effects of dietary CP concentrations greater than 15.5% in combination with protein degradability. In finishing cattle diets with excess CP, protein degradability may play a role in animal performance because of potential metabolic costs associated with ammonia detoxification (Lobley et al., 1995).

The development of new technologies has changed the way cattle are managed and marketed in the finishing cattle industry. For example, the use of beta-agonists such as ractopamine hydrochloride **(RH)** and zilpaterol hydrochloride **(ZH)** for the last 20 to 42 d of the finishing period has been shown to increase animal performance and muscle deposition (Avendaño-Reyes et al., 2006; Arp et al., 2014). Because beta-agonists increase N retention, it is likely these feed additives influence protein metabolism (Reeds and Meresmann, 1991). Although the interaction between dietary protein and beta-agonists has already been investigated (Walker et al., 2006; Samuelson et al., 2014), these studies did not determine how cattle receiving beta-agonists would respond to concentrations of dietary CP greater than those provided in most finishing diets. Therefore, the objective of this study was to determine the effects of excess dietary CP, provided primarily as either RDP or RUP, on performance and carcass characteristics of cattle consuming diets with or without RH.

MATERIALS AND METHODS

Receiving Cattle Management

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Approximately 10 mo before initiation of the experiment, crossbred heifers (187 ± 0.6 kg BW) from south Texas were received at the Clayton Livestock Research Center. Heifers were divided between two 56-d receiving studies (Oosthuysen et al., 2015). At initial processing, calves were weighed using a Daniels Bud Box System (AH-10; Daniels Mfg, Ainsworth, NE), vaccinated, and provided with oral parasiticide (Safeguard; Intervet Inc., Millsboro, DE). After

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these receiving studies were completed, all calves were limit-fed once daily with approximately 11.4 kg of a commercially available starter diet (RAMP; Cargill Inc., Dalhart, TX).

Experimental Design and Dietary Treatments

In May 2015, 525 heifers were weighed individually (423 ± 1.8 kg BW) and ranked according to BW. Heifers were then separated into 4 blocks based on BW and assigned consecutively to pens so that the average weight and standard deviation was similar among pens. After initial BW were recorded, heifers were sorted into their respective blocks, and then sorted into the appropriate pen. At this time, heifers were re-vaccinated (Vista 3; Merck Animal Health, Summit, NJ) and given a commercial growth implant (Revalor-200; Merck Animal Health).

The study was a randomized complete block design consisting of 48 soil-surfaced pens (12 × 35 m, with 11 m bunk line) and 4 blocks (12 pens per block with 9 to 11 heifers per pen). Within each block, pens of cattle were randomly assigned to 1 of 6 dietary treatments in a 2 × 3 factorial arrangement. Treatments consisted of either RH (Actogain; Zoetis, Florham Park, NJ) or no RH supplied to cattle consuming 1 of 3 dietary protein treatments. Protein treatments (Table 1) were steam-flaked corn-based diets containing 13.9% CP, 8.8% RDP, and 5.0% RUP (**CON**), 20.9% CP, 13.4% RDP, 6.1% RUP (**High RDP**), or 20.9% CP, 9.1% RDP, 10.4% RUP (**High RUP**). The dietary treatments were initiated after pens of cattle were weighed (initial BW = 498, 527, 551, and 575 ± 3.82 kg for block 1, 2, 3, and 4, respectively), 35 d before harvest (treatment period).

For the entire 35-d treatment period, cattle were fed twice daily and RH treatments were top-dressed directly onto protein treatments in feed bunks once daily to supply either 0 or 400 mg of RH per heifer. For pens receiving the RH treatment, the appropriate amount of RH for a pen of cattle was mixed with 150 g wet corn gluten feed (Sweet Bran; Cargill Inc.), whereas pens receiving no RH were top-dressed with 150 g of wet corn gluten feed only. After top-dressing, RH treatments were mixed with the ration in the bunk using a rake in an attempt to evenly distribute the RH treatments with the treatment diets.

Feeding Management and Collections

The amount of feed to offer each pen was determined by visually evaluating feed bunks twice daily, with the bunk management protocol designed to leave little to no accumulation of feed at the next feeding. Each diet was mixed in an overhead ribbon mixer (Hayes & Stolz Industrial Mfg. Co, Fort Worth, TX) immediately before feeding and delivered to cattle using a 6-compartment feed truck with individual dispensing augers. Both individual ingredient and mixed ration samples were collected weekly for analysis of DM (100°C for 48 h in a forced-air oven) and other nutrients (Servi-Tech Laboratories; Amarillo, TX). Any refusals or feed remaining as a result of inclement weather were collected and analyzed for DM to calculate daily DMI.

Once all cattle within a block had received the appropriate RH treatment for 35 d, they were weighed as a group using a pen scale before the morning feeding and shipped to a commercial slaughter facility (Cargill Meat Solutions, Friona, TX). Once received at the processing plant, heifers were harvested and HCW and liver scores were recorded. Following a 24 h chill, individual carcass measurements were recorded and yield grade values were calculated. All parameters reported for carcass characteristics were collected by personnel from the Beef Carcass Research Center (West Texas A&M University, Canyon, TX).

Initial and final BW were adjusted with a 4% shrink and dressing percentage was calculated by averaging the HCW of heifers in each pen, and dividing by the average final BW of heifers in that pen. Carcass-adjusted performance data was calculated using a final BW determined by dividing the HCW by a common dressing percentage (64%). All performance parameters presented were calculated on a dead's out basis as 1 animal died during the study due to causes unrelated to treatment.

Statistical Analysis

For all performance and carcass variables measured, pen served as the experimental unit. Performance and carcass data with continuous variables were analyzed using the MIXED procedure (SAS Inst.; Cary, NC) with initial BW as a covariate in the model. Nominal data such as liver scores and quality grades were analyzed using the GLIMMIX procedure. For all statistical analysis the model included diet, RH, and the interaction between diet and RH. Block served as the random effect, with the experiment blocked according to BW of the cattle, which subsequently determined the date for both initiation of RH treatments and harvest. Treatment differences were considered significant when $P \leq 0.05$ and a tendency when $0.05 < P \leq 0.10$.

RESULTS

Interaction between Dietary Protein and RH

No dietary protein × RH interactions ($P \geq 0.39$) were observed for performance (Table 2). Similarly, dietary protein × RH interactions ($P \geq 0.11$) did not occur for any of the carcass parameters.

Effects of Dietary Protein

Final BW and ADG were not different ($P = 0.12$) among treatments (Table 2). Both carcass-adjusted final BW and ADG for cattle receiving High RDP tended to be greater ($P = 0.06$) than those receiving High RUP or CON, and not different between cattle receiving High RUP and CON. Dry matter intake, water intake, G:F and carcass-adjusted G:F were not different ($P = 0.12$) among the 3 dietary protein treatments. Hot carcass weights tended to be greater ($P = 0.06$) for cattle receiving High RDP compared to High RUP and CON; HCW were not different between cattle receiving High RUP and CON. Dressing percentage was lower ($P < 0.01$) for cattle fed High RUP than High RDP and CON,

and not different between cattle receiving High RDP and CON. Marbling score, 12th rib fat depth, LM area, and yield grade were not different ($P \geq 0.16$) among dietary protein treatments. Heifers receiving High RUP tended to have lower ($P = 0.10$) KPH than heifers receiving CON, and were not different among heifers receiving High RDP and CON or High RDP and High RUP. The percentage of carcasses grading USDA choice tended to be greater ($P = 0.09$) for heifers receiving High RDP compared to High RUP; no differences were observed between both High RDP and CON and High RUP and CON for the percentage of carcasses grading choice. The percentage of liver abscesses was not different ($P = 0.94$) among dietary protein treatments.

Effects of RH

Final BW and carcass-adjusted final BW of heifers receiving RH were greater ($P < 0.01$) than those not receiving RH. Cattle receiving RH had greater ($P < 0.01$) ADG and carcass-adjusted ADG and similar ($P \geq 0.36$) DMI and water intake compared with cattle not receiving RH. This resulted in a greater ($P < 0.01$) G:F and carcass-adjusted G: F for cattle fed RH than those that were not fed RH. Hot carcass weights were greater ($P < 0.01$) and dressing percentage tended to be greater ($P = 0.09$) for cattle receiving RH, while marbling score was not affected ($P = 0.11$) by RH treatment. Measurements for 12th rib fat depth tended to be lower ($P = 0.08$), and KPH was lower ($P = 0.02$) for cattle receiving RH vs. no RH. Longissimus muscle area was greater ($P = 0.03$) for cattle receiving RH compared with cattle that did not receive RH. No difference ($P \geq 0.12$) was observed in yield grade or quality grade for cattle consuming RH compared to no RH. The incidence of liver abscesses was not different ($P = 0.14$) among RH treatments.

DISCUSSION

Interaction between Dietary Protein and RH

Because beta-adrenergic agonists increase protein deposition, N retention, and uptake of AA (Beerman et al., 1993; Byrem et al., 1998), we hypothesized that RH may alter cattle requirements for CP, and therefore would decrease potential negative effects of excess dietary CP on performance. However, no dietary protein × RH interactions suggest that RH did not influence CP requirements of finishing cattle. Walker et al. (2006) reported that cattle receiving RH did not benefit from additional MP (provided primarily by RUP) in the diet, suggesting that the amount of MP in diets typically fed to finishing cattle is adequate to meet CP requirements for cattle receiving beta-agonists. Samuelson et al. (2014) indicated that performance of cattle receiving ZH was not improved by increasing RDP above the 8.3% recommended for steam-flaked corn-based finishing diets by Cooper et al. (2002). These results indicated that although protein deposition increased with beta-agonists, alterations in protein metabolism were not large enough to change overall protein requirements or influence the need for degradable vs. undegradable protein.

Effects of Dietary Protein

Dietary protein supplied in excess of animal requirements may increase AA oxidation and NH_3 detoxification (NRC, 1985). Amino acid catabolism in the liver and detoxification of NH_3 absorbed from the gastrointestinal tract might be associated with an energy and amino acid cost, as demonstrated by increased oxygen and AA consumption (Lobely et al., 1995). Therefore, we hypothesized that excess dietary CP could negatively impact performance and carcass merit of finishing cattle. However, ADG, DMI, and G:F were not different among cattle receiving CON, High RDP, and High RUP, suggesting that excess dietary CP, regardless of ruminal degradability, did not negatively affect performance in the present study. This agrees with previous research completed at lower CP concentrations, whereby performance of cattle was not further improved by providing greater than 8.2% RDP (Gleghorn et al., 2004) or 5.1% RUP (Wagner et al., 2010) in steam-flaked corn-based finishing diets. Because excess CP (provided either as RDP and RUP) did not negatively affect any of the performance or carcass variables measured in the present study, it seems that cattle were able to efficiently clear excess N-containing compounds from the body without greatly increasing energy costs.

Effects of RH

Greater live performance is commonly associated with RH administration in finishing cattle (Avendaño-Reyes et al., 2006; Bryant et al., 2010). In the present study, heifers receiving RH had greater final BW (1.9% increase), ADG (33.4% increase), and G:F (34.4% increase) and greater carcass-adjusted final BW (2.4% increase), ADG (42.9% increase) and G:F (44.8% increase) than heifers not receiving RH. The results observed for ADG are greater than the 16% increase reported by Abney et al. (2007) for cattle receiving 200 mg RH daily, however, their study indicated that the ADG response linearly increased with dose, and therefore is consistent given that 400 mg RH (2 fold greater) was used in the present study. Research by Quinn et al. (2008) and Avendaño-Reyes et al. (2006) suggests that providing greater concentrations of RH decreases DMI. However, decreased DMI was not observed in the current study or by Arp et al. (2014), who also fed 400 mg RH per animal daily. These variations in DMI could be a result of differences in cattle feeding and management decisions or days on feed among studies.

Hot carcass weights were greater for heifers that received RH. Increases in HCW from 5.3 to 13.6 kg have been observed with RH (Avendaño-Reyes et al., 2006; Scramlin et al., 2010), however, HCW increases greater than 8 kg only occurred when cattle were provided with 300 mg RH daily or greater, which is consistent with the 8.5 kg increase in HCW observed in the current study. In contrast to Gruber et al. (2007) and Arp et al. (2014), dressing percentage of cattle receiving RH was slightly greater (0.39% increase)

than those not receiving RH. However, this is most likely a function of RH dosage as Arp et al. (2014) reported that dressing percentage was greater for cattle receiving 300 and 400 mg RH per day than those receiving 200 mg. Marbling score was not affected by RH, which agrees with Bryant et al. (2010). Alternatively, decreased 12th rib fat depth and KPH indicate that RH may have more directly affected body fat composition in the current study than in previously reported research. Cattle fed RH had 1.76 cm^2 greater LM area, which is indicative of increased muscle deposition. No difference in yield grade, quality grade, and liver abscesses agree with previously reported data, although many of these studies did not provide information on the incidence of liver abscesses relative to RH. In conclusion, the results observed for RH administration in the present study agree with previously reported research, but also suggest that dosage and length of administration may play an important role in the results observed for RH. In particular, providing higher doses of RH may result in larger improvements in performance and have greater effects on traits relative to carcass characteristics.

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Table 1. Ingredients and nutrient composition of diets fed to finishing heifers

	Treatments[1]		
Item	CON	High RDP	High RUP
Ingredient, % of DM			
Corn grain, flaked	67.1	57.0	57.0
Wet corn gluten feed[2]	18.0	18.0	14.5
Corn stover	9.00	9.00	9.00
Soybean Meal	-	9.60	-
Corn gluten meal	-	-	14.5
Corn oil	0.90	1.40	-
Urea	1.01	1.49	-
Supplement[3]	3.99	3.51	5.00
Nutrient Analysis[4], DM basis			
CP, %	13.9	20.9	20.9
RDP, %	8.82	13.40	9.05
RUP, %	5.02	6.05	10.44
NE_m, Mcal/kg	2.26	2.28	2.28
NE_g, Mcal/kg	1.56	1.58	1.58

[1]Treatments were in a 2 × 3 factorial arrangement with 2 levels of ractopamine hydrochloride and 3 dietary protein treatments.
[2]Sweet Bran (Cargill Inc., Dalhart, TX).
[3]Contained dried distillers grains with solubles, limestone, salt, trace minerals (1.8% Cu, 9.0% Zn, and 360 ppm Se; Beefmax 0510; Cargill Inc.), vitamins (1030 IU vitamin A, 500 IU vitamin D, and 5.62 IU vitamin E per kg of DM), and medicated supplement (supplied 33 mg of monensin and 9.8 mg of tylosin per kg of dietary DM; Elanco Animal Health, Indianapolis, IN).
[4] Nutrient concentrations are based on laboratory analysis (Servi-Tech Labs, Amarillo, TX).

Table 2. Effects of ractopamine hydrochloride (RH) and dietary protein concentration and degradability on performance and carcass characteristics of finishing heifers

	Treatments[1]							*P*-value[2]		
	No RH			RH						
Item	CON	High RDP	High RUP	CON	High RDP	High RUP	SEM	Diet	RH	Diet × RH
Performance										
Initial BW[3], kg	541	547	526	541	546	527	-	-	-	-
Final BW, kg	571	572	572	581	582	586	2.03	0.12	<0.01	0.42
Adj. final BW[4], kg	569	571	567	581	586	581	2.25	0.06	<0.01	0.80
ADG, kg	0.931	0.967	0.979	1.22	1.26	1.36	0.06	0.12	<0.01	0.42
Adj. ADG[5], kg	0.885	0.949	0.836	1.23	1.36	1.22	0.06	0.06	<0.01	0.80
DMI, kg/d	9.06	9.35	9.11	9.06	9.14	9.17	0.12	0.28	0.57	0.46
Water Intake, L/d	30.1	30.8	34.2	32.6	31.8	33.9	2.13	0.19	0.36	0.59
G:F	0.103	0.105	0.106	0.136	0.138	0.147	0.007	0.27	<0.01	0.39
Adj. G:F[6]	0.097	0.102	0.090	0.137	0.149	0.133	0.008	0.12	<0.01	0.81
Carcass										
HCW, kg	364	366	363	372	375	372	1.44	0.06	<0.01	0.80
Dressing percent	63.8	63.9	63.4	64.0	64.4	63.5	0.20	<0.01	0.09	0.51
Marbling score	44.2	43.6	44.3	42.2	44.4	42.2	1.21	0.62	0.11	0.16
12^{th} rib fat, cm	1.61	1.75	1.49	1.51	1.55	1.52	0.09	0.16	0.08	0.23
LM area, cm^2	91.5	92.2	91.9	93.9	94.6	92.3	1.17	0.48	0.03	0.51
KPH, %	3.50	3.33	3.09	3.14	3.15	3.11	0.15	0.10	0.02	0.11
Yield grade	2.61	2.72	2.44	2.45	2.48	2.52	0.13	0.54	0.14	0.18
Choice, %	51.4	55.2	45.4	47.9	56.8	42.5	5.37	0.09	0.72	0.87
Liver abscesses, %	10.53	9.20	6.90	4.21	4.55	7.96	3.52	0.94	0.14	0.37

[1]Treatments were a 2 × 3 factorial arrangement with 2 levels of RH and 3 dietary treatments (CON = finishing diet containing 13.9% CP, 8.82% RDP, 5.02% RUP; High RDP = finishing diet containing 20.9% CP, 13.4% RDP, 6.05% RUP; High RUP = finishing diet containing 20.9% CP, 9.05% RDP, 10.44% RUP).
[2] Diet = *P*-value for main effect of diet; RH = *P*-value for the main effect of RH; Diet × RH = *P*-value for the interaction of diet and RH.
[3]Initial BW was not statistically analyzed as a response variable because it was used a covariate in the analysis of continuous data for performance and carcass characteristics.
[4]HCW adjusted to a common dressing percentage of 64.0%.
[5]Carcass adjusted Final BW – Initial BW / 35 d.
[6]Carcass adjusted ADG / DMI.

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Evaluation of *Eragrostis tef* (Zucc.) as a forage option for grazing beef cattle in the Southern High Plains

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ABSTRACT: In order to assess the potential of *Erogrostis tef* to provide a late summer supply of forage for livestock production in the Southern High Plains, four 2.66 ha paddocks equipped with subsurface drip irrigation were seeded with *Eragrostis tef* ('Tiffany' teff) at a rate of 3.72 kg ha^{-1}. Each paddocks was stocked with commercial beef steers (n = 5; 289 ± 30.38 kg initial shrunk BW) at 51 d post-seeding resulting in an initial forage allowance of 202 kg DM/100 kg BW. Weekly samples of whole plant and canopy structure were obtained to describe DM, OM, and fiber concentrations. Appropriate sample height of canopy for the purposes of estimating selection quality was determined at the most proximal grazing site to each quadrat toss by recognition of a tiller with at least one leaf possessing the flat defoliation pattern characteristic of an ungulate bite. Biweekly samples included analysis of CP and IVTD. Leaf percentage of entire plant was quantified at 21 d intervals. Available DM and OM peaked at day 28 and was lowest at day 56 (P < 0.01). Whole plant and canopy DM (P < 0.01), OM (P < 0.01), NDF (P < 0.01), ADF (P < 0.01), and CP (P < 0.01) differed by day. Only *in vitro* true digestibility was not affected by maturation of either whole plant (P = 0.12) or canopy structure (P = 0.61). Leaf proportion of whole plant structure aligned with times of peak forage mass availability (P < 0.01). Teff grass stocked at a moderate rate with growing beef calves achieved adequate production with minimal inputs forage with minimal inputs to provide a quality forage base for approximately two months of grazing in the Southern High Plains.

Key words: digestibility, forage quality, grazing, teff, water[1]

INTRODUCTION

Production of forage and livestock in the Southern High Plains relies partially on the increasingly depleted Ogallala Aquifer as the water source for irrigation and stock watering. Regional late-summer growth and quality of forages is often poor due to climate factors including high rates of evapotranspiration, sporadic rainfall, and warm air temperatures. As a result, enterprises with irrigation capacities often face production decisions which will either further consume subsurface water or lead to reduced annual production due stalled forage growth. Teff [*Eragrostis tef* (Zucc.) Trotter] is an annual, warm-season grass which has the potential to enhance agronomic productivity. Relative to more conventional annual warm-season forages, early research with teff has indicated potential for high DM yield, vigor, and forage quality (Hunter et al., 2008). Additionally, water and nutrient inputs associated with establishing teff a stand are minimal by comparison and observations of rapid establishment and growth rate contribute to its potential as an emergency forage source or an intermediate crop in rotation (Roseburg et al., 2005; Hunter et al., 2007; Young et al., 2014). Local adaptation, resource inputs, and growth potential are strong drivers of forage yield whereas forage quality ultimately determines the value of forage in livestock production. Beaty and Engel (1980) identified analysis of constituents influencing forage intake and digestibility as highly pertinent criteria for evaluating forages for livestock production. Regulation of nutrient provision by the dynamic association of canopy structure and plant maturity suggest that periodic sward sampling might be an effective means of tracking changes in forage quality. The substantial majority of literature regarding teff quality and water use has been produced internationally where teff grain is a primary source of carbohydrate in human nutrition. Information regarding teff primarily as grazing forage is limited and estimates of quality trends under continuous grazing are lacking. Thus, objectives of this study were to discern responses of teff quality and herbage mass under continuous grazing management. These data are part of a larger effort to estimate water requirements by teff in a high-evaporative-demand environment and as a possible forage option for dryland or ultra-low irrigation in an integrated crop-livestock system.

MATERIALS AND METHODS

All procedures involving the use of animals were conducted under an approved Animal Care and Use Protocol.

Site Description

On June 11 and 12, 2015, 10.62 ha equipped with subsurface drip irrigation at the Texas Tech University Research Farm at New Deal were seeded with *Eragrostis tef* ('Tiffany' teff) at a rate of 3.72 kg ha^{-1} pure live seed using a no-till drill into a Pullman clay loam soil. Seedbeds were prepared prior to seeding and no fertilizer or herbicide was applied. Preceding data collections, the seeded area was equally into four 2.66-ha paddocks.

Each paddock was stocked with commercial beef steers (n = 5; 289 ± 30.38 kg initial shrunk BW) in order to

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estimate effects of grazing and potential structural preference by cattle on regrowth. Steer BW was obtained following a 12-h fast at day zero and each 21 d thereafter for the purposes of monitoring response to forage availability and quality. Steers were placed on paddocks beginning July 31 and removed October 2 following 63 days of grazing. Initial stocking rate was 1.9 steers/ha equating to an individual forage allowance of 202 kg DM/100 kg shrunk BW.

Forage and Field Collections

Each week, six 0.25 m^2 quadrats were randomly tossed within each paddock and forage within clipped at a height of 3.0 cm to determine DM, OM, NDF, and ADF. At biweekly intervals beginning d 14, canopy structure was sampled to estimate the magnitude of difference in total available nutritive value and that of the apparent preferred fraction. Estimation of appropriate depth from which canopy samples were obtained was determined at the most proximal grazing site to each quadrat toss by identification of a tiller with at least one leaf possessing the flat defoliation pattern characteristic of an ungulate bite. Nearest entire plant structures were clipped at a level visually proportionate to the adjacent grazed level. Both whole canopy and apparently grazed samples were dried in a forced air oven (55°C) for 72 h and ground to pass a 1-mm screen through a Wiley mill (Thomas Scientific, Swedesboro, NJ). In addition to DM, OM, NDF, and ADF, forage samples from quadrats were used for biweekly estimates of available forage mass as well as CP concentration and *in vitro* true digestibility. Within each paddock, an additional sampling of whole plant structures was randomly collected from 12 locations every 21 d for partitioning leaf and stem fractions.

Volumetric soil water content was determined using a PR2 Soil Moisture Profile Probe (Delta-T Devices, Cambridge, UK) at depths of 100, 200, 300, 400, 600, and 1000 mm at three evenly spaced locations spanning each paddock.

Chemical Analysis and Digestibility

All procedures were conducted in duplicate. Ground forage samples were dried in a forced-air oven at 100°C to determine laboratory corrected DM. Organic matter was calculated from ash residue remaining after complete combustion in a muffle furnace at 550°C for 8 h (AOAC, 1990). Crude protein was determined using a Leco CNS Nitrogen Analyzer (Leco CNS-200, St. Joseph, MI). Neutral detergent fiber and ADF were derived using filter bag techniques (Ankom Technology, 2006a,b). *In vitro* true digestibility (IVTD) measures were obtained by Daisy^{II} Incubator (Ankom Technology, 2005).

Statistical Analysis

Forage mass, nutrient composition, IVTD, leaf percentage, and soil water data were analyzed to determine differences by day using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC) with paddock as experimental unit. When the *P*-value of the *F*-statistic were ≤ 0.05, least squares mean differences within day were separated by Tukey's HSD test and declared significant at $\alpha=0.05$.

RESULTS AND DISCUSSION

Forage allowance was greatest at turnout and declined as the trial progressed to 110 kg DM/100 kg BW at the conclusion. These results justified analysis of both seemingly complete and truncated specimens as a decrease in forage allowance would theoretically reduce the opportunity for preferential grazing and thereby alter diet composition.

Body weight increases by steers throughout the trial period did not influence stocking rate if paddock. However, BW gain during the middle third of the experiment was higher than initial and terminal phases (data not shown). These trends may be at least partially explained by forage availability in that a corresponding pattern of DM mass was observed across sampling days (Table 1). Capacity for teff regrowth is expected to be mitigated when height is reduced to less than 8 cm (Roseberg et al., 2015). However, in the current study, grazing pressure did not result in visual observations of depletion below that critical level.

Dry matter yields for teff in the present study were less than previously observed (Hunter et al., 2007; Lemus et al., 2009). Reduction from previously reported studies should likely not be attributed to inadequate seeding rate as multiple studies have reported rates as low as 2 kg/ha without sacrifice of total DM yield (Roseberg et al., 2005; Hunter et al., 2007). In these trials, the initial measure was observed no later than 61 days, a duration of ten post-seeding days beyond that of this study. However, neither of the referenced experiments defined seed as bulk or pure live seed. Hunter et al. (2009) suggested that teff seeding rate ranges from 4.5 to 8.0 kg/ha. While abstention from fertilizer application in this study was likely a partial contribution to reduced yield, results of others indicate that both applied moisture and nitrogen may result in diminished or otherwise stagnant production when levels exceed a yield-response plateau. In regards to nitrogen, this value has been proposed as 67 and later 102 kg ha^{-1} of nitrogen (Roseberg et al., 2005; Hunter et al., 2008).

Teff has reportedly produced robust stands in soil moisture conditions ranging from drought-stressed to water-logged. However, genotype of cultivar may contribute to growth potential (Degu et al., 2008). Mean soil water measures were 11, 25, 28, 41, 44, and 45% at depths measured, respectively. The deepest three depths (40, 60, and 100 cm), with values exceeding 40% water content, would indicate adequate water to sustain growth in the deeper profile. Soil moisture requirements by depth are not fully elucidated and root depth of teff in this study was not quantified.

Crude protein measures observed in this study ranged from 4.25 to 7.31% (Table 1). These values are lower than those observed elsewhere. Numerous estimates of teff CP concentration reflect scenarios of vegetative or early boot stages and nitrogen fertilization. These studies have estimated teff CP ranges of 15 to 17% (Hunter et al., 2007; Lemus et al., 2009). In both of these cases, yields also far exceeded what is found in literature, approaching 9,000

kg/ha. Differences in whole plant structure and the apparently grazed canopy fraction in this trial accounted for an increase in CP of 1.1 percentage points. Little evidence of other comparable data regarding morphologically specific values could be located in published literature. However, teff is often characterized as having a small, thin stem and generous leaf area. Whereas this description does not contribute to an explanation for lower CP concentration and is inconsistent with expectations of greater leaf mass, a lesser improvement in CP solely from plant canopy analysis should be easier to elucidate considering the subtlety of difference overall structure.

Reduction in leaf proportion of the full plant structure, relative to the apparently grazed fraction, was most prevalent in the initial 21 d of experiment. Intermediate measures were similar with a mean of 40.67%. These results explain the decline in whole plant CP which was greatest during the same time frame. Canopy measures were not obtained on day of initiation as grazing had not commenced. However, leaf percentage values reaffirm that leaf florescence CP concentration of teff.

Detergent fiber fractions of teff often range from near 60 to 65% and 35 to 40% for NDF and ADF, respectively. Measures in this study were higher among whole plant samples and, as expected, increased with advancing maturity. These data suggest that leaf tissue was available in adequate amounts throughout the trial to support at least the majority of forage selected. Mean ADF of complete plant sample was 33.11 % whereas the apparent selected fraction was comprised of 32.85%. These values were lower relative to published estimates of 35 to 40% . Mean ADF of whole plant material was 33.1%, and canopy tissues produced a similar mean of 32.8%. over the trial duration. As a proportion of increase with age, whole plant fractions increased 25.1% from initial to final measure, whereas apparently grazed canopy estimates increased 16.0%.

Decreases in *in vitro* true digestibility were similar over the sequence of daily observations for whole plant and abridged structures. Trial means were 57.4% and 55.9%, respectively. A proportional reduction in ADF of apparent selection samples correctly predicted a lesser reduction in digestibility relative to whole plant results. Maturation over the late summer reduced digestibility of the full plant structure by 6.63% whereas reduction in estimated grazed canopy mass was tempered 2.2%.

IMPLICATIONS

Teff grass established quickly and when stocked at a moderate rate with growing beef calves achieved adequate production with minimal inputs to provide a forage base for roughly two months in the Southern High Plains. Prior studies of teff which have included fertilizer and water applications at a range of specified rates and reported increases in available forage mass and CP values relative to this experiment where

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Table 1. Forage mass and comparative analysis by sampling day of whole plant and canopy structure of teff *Erogrostis tef* (Zucc.) grazed continuously.

	Day											
Item	0	7	14	21	28	35	42	49	56	63	SE	*P*
Forage availability, kg/ha												
DM	1095.8^{b}	--	1650.6^{a}	--	1762.3^{a}	--	1258.1^{ab}	--	806.1^{b}	--	115	0.01
OM	1001.6^{c}	--	1504.1^{ab}	--	1619.2^{a}	--	1148.1^{bc}	--	729.4^{c}	--	105	0.01
Leaf proportion, % total plant weight												
	48.1^{a}	--	--	39.5^{ab}	--	--	41.8^{ab}	--	--	42.2^{b}	1.63	0.02
Whole plant chemical composition, %												
DM	94.8^{a}	93.0^{b}	91.7^{d}	92.3^{bcd}	92.5^{bc}	92.6^{b}	92.4^{bc}	91.8^{cd}	92.9^{b}	--	0.15	0.01
OM	91.3^{ab}	90.8^{abc}	91.1^{ab}	91.1^{ab}	91.8^{a}	91.0^{abc}	91.0^{abc}	89.8^{c}	90.3^{bc}	--	0.27	0.01
NDF	66.1^{c}	70.8^{b}	70.5^{b}	70.0^{b}	70.4^{b}	70.6^{b}	68.7^{b}	70.9^{b}	74.9^{a}	--	0.64	0.01
ADF	29.2^{d}	32.7^{c}	32.6^{bc}	32.6^{bc}	32.0^{cd}	33.0^{bc}	33.9^{abc}	35.4^{ab}	36.6^{a}	--	0.59	0.01
CP	7.31^{a}	--	5.5^{b}	--	4.9^{bc}	--	5.0^{bc}	--	4.25^{c}	--	0.21	0.01
IVTD	61.3^{a}	--	57.5^{a}	--	56.5^{a}	--	54.8^{a}	--	57.2^{a}	--	1.27	0.12
Apparently grazed chemical composition, %												
DM	--	93.4^{ab}	92.4^{c}	92.6^{bc}	92.6^{bc}	92.7^{bc}	92.1^{c}	92.2^{c}	93.9^{a}	--	0.21	0.01
OM	--	91.1^{a}	88.7^{b}	88.7^{b}	87.6^{b}	87.0^{b}	87.0^{b}	87.2^{b}	87.0^{b}	--	0.49	0.01
NDF	--	68.7^{b}	68.7^{b}	67.0^{b}	66.2^{b}	67.9^{b}	64.2^{b}	66.6^{b}	69.7^{b}	--	1.30	0.01
ADF	--	30.7^{c}	32.3^{bc}	32.3^{bc}	32.8^{bc}	32.2^{bc}	33.8^{ab}	33.2^{abc}	35.6	--	0.57	0.01
CP	--	--	7.2^{a}	--	5.7^{b}	--	5.5^{b}	--	5.7^{b}	--	0.18	0.01
IVTD	--	--	57.3^{a}	--	54.1^{a}	--	56.0^{a}	--	56.0^{a}	--	1.31	0.61

^{a,b,c}Means within row without common superscript differ at $P \leq 0.05$.

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Salivary cortisol concentrations affect rumen microbial fermentation and nutrient digestibility in vitro

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ABSTRACT: This study investigated effects of cortisol on fermentation and digestibility of nutrients by rumen microorganisms. Four dual-flow continuous culture fermentor (2,700 mL) systems were used in a 4 x 4 Latin square design with 4 periods and 4 treatments. Each experimental period consisted of 13 d, which allowed 7 d for microbial adaptation, 3 d for cortisol treatment application, and a 3 d post-treatment period. Treatments consisted of 0, 3, 6, or 9 ng/mL of cortisol mixed into artificial saliva and continuously infused at a rate of 1.55 ± 0.05 mL/min. For the 3 d treatment period and the 3 d post-treatment period samples were collected at 0, 3, 6, 9, 12, 24, 36, 48, 60 and 72 h and were analyzed VFA, and NH_3. During the 3-d sampling period effluent was composited for analysis of OM, NDF, and CP digestibility. During cortisol treatment, cortisol × h ($P \leq 0.01$) was observed for acetate and valerate, total VFA and isobutyrate tended to increase from 0 to 3 ng/mL, but were not different among 3, 6, and 9 ng/mL (quadratic, $P \geq 0.12$), butyrate tended to decrease from 0 to 3 ng/mL cortisol, and was not different among 3, 6, and 9 ng/mL (quadratic, $P = 0.07$), and isovalerate was not different among 0, 3, and 6 ng/mL, but decreased from 6 to 9 ng/mL of cortisol (quadratic, $P = 0.06$). Digestibility of OM (g/d and % of intake) tended to be lower (quadratic, $P = 0.12$), and NDF digestion (g/d and % of intake) was lower (quadratic, $P = 0.09$) for 9 ng/mL cortisol compared to 0, 3, or 6 ng/mL. Digestibility of CP (g/d and % of intake) was not different ($P \geq 0.23$) among treatments. In the period after cortisol treatment, a cortisol × h ($P = 0.03$) was observed for isobutyrate, NH_3 decreased linearly ($P = 0.04$) with increasing cortisol, valerate tended to increase linearly ($P = 0.14$) with increasing concentrations of cortisol, and isovalerate was lower for 9 ng/mL than 0, 3, and 6 ng/mL cortisol (quadratic, $P = 0.10$). Digestibility (g/d and % of intake) of OM, NDF, and CP were not different ($P \geq 0.51$) among treatments. Results indicate that cortisol may influence rumen microbial fermentation and digestion when present in saliva at 9 ng/mL.

Key words: cortisol, continuous flow fermentors, digestibility, fermentation, rumen microbes, stress

INTRODUCTION

Animals exposed to stressful conditions exhibit acute increases in blood and salivary concentrations of catecholamines (Freestone and Lyte, 2010) and glucorticoids (Yates et al., 2010), which may negatively affect animal performance either directly (Foote et al., 2016) or indirectly because of loss of appetite (Hutcheson and Cole, 1986). Increased stress hormones can also be indicative of positive animal welfare states, as these hormones help to regulate energy metabolism, immune function, and overall fitness (Ralph and Tilbrook, 2016).

Research in ruminants indicates exposure to a stress event such as fasting and transportation (Galyean et al., 1981) or an endotoxin challenge (Gilliam et al., 2009) may alter rumen function. This could be particularly important for immune function of nutritionally compromised animals, as changes in microbial fermentative and digestive capacity could shift time required for animals to recover from stress. Although altered rumen function could, in part, be attributed to changes in DMI or passage rates associated with stress, it is also possible that rumen microorganisms respond directly to mammalian stress hormones.

In a review, Freestone and Lyte (2010) indicated that growth of pathogenic bacteria found within the gastrointestinal tract increases with exposure to catecholamines. Furthermore, research conducted by Tajima et al. (2007) indicated that rumen bacterial populations may respond to host signals from animals subjected to heat stress. We hypothesized that increasing concentrations of salivary cortisol would alter ruminal fermentation and microbial digestion. Therefore, the objective of this study was to determine the effects of cortisol on rumen fermentation characteristics and digestibility of nutrients of rumen microorganisms in dual-flow continuous culture fermentors.

MATERIALS AND METHODS

Experimental Design and Fermentation Conditions

Four dual-flow 2,700 mL continuous culture fermentor systems (Bioflo 115; Eppendorf North America, Hauppauge, NY) were used in a 4 × 4 Latin square design with 4 periods and 4 treatments to determine the effects of cortisol on rumen fermentation. Each experimental period consisted of 13 d, which allowed 7 d for microbial adaptation to the fermentor system, 3 d for cortisol treatment application, and a 3-d post-treatment period. On d 1 of every 13-d fermentation, approximately 1 L of ruminal fluid was collected from each of 3 ruminally-cannulated crossbred cows (BW = 453 ± 25 kg) using a polypropylene vacuum flask and suction strainer (Precision Machine Co, Lincoln, NE). Donor cattle were fed a 41% (DM basis) cracked corn-based diet (Table 1) at 1.7% of BW for a minimum of 7 d before collection of rumen contents. After collection, ruminal fluid was transported to the laboratory

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in a preheated (±39°C) thermos to provide approximately 3 L of mixed ruminal fluid to inoculate the fermentors. At initiation of each 13-d fermentation, each glass fermentation vessel was inoculated with 500 mL of mixed ruminal fluid and 500 mL artificial saliva (Weller and Pilgrim, 1974).

Each fermentor was equipped with an internal controller (Eppendorf North America) and external pump (120U; Watson-Marlow, Cornwall, UK) to maintain pH (5.0 to 8.0 ± 0.5 by infusion of either 5 *M* NaOH or 0.3 *M* H_2SO_4). Heat jackets covering the fermentation vessels maintained temperature (±39°C) of ruminal fluid, which was continuously stirred as described by Lodge-Ivey et al. (2010). Anaerobic conditions were maintained by infusion of N_2 at 40 mL/min. To simulate recycled N, artificial saliva containing urea (0.5 g/L) was continuously infused at 1.55 ± 0.05 mL/min. Fermentor effluent was removed via filtered suction strainer (Bar Diamond Inc., Parma, ID) at 1.0 mL/min, and each morning the vessels were returned to starting capacity (1 L) by pumping out effluent to maintain solid (5%/h) and liquid (10%/h) flow rates as described by Lodge-Ivey et al. (2010). Feed (± 66 g of DM; Table 1) was divided evenly and fed twice daily to each fermentor. Before feeding, basal dietary ingredients, salt, and urea were ground in a Wiley Mill (Wiley Mill Thomas Scientific, Swedesboro, NJ) to pass a 1 mm screen, mixed with limestone and molasses in a commercial mixer (150F; Lasar Mfg. Co., Los Angeles, CA) and pelleted using a laboratory-scale pellet mill (Eco3; Colorado Mill Equipment, Colorado Springs, CO). Pellets were then dried in a 55°C forced-air oven (Blue M Electric Co., Blue Island, IL) for 24 h.

Treatment Arrangement and Application

Treatments were 4 concentrations of cortisol in artificial saliva. Hydrocortisone powder (Sigma Aldrich, St. Louis, MO) was mixed with ethyl alcohol (200 proof; Pharmco-aaper, Brookfield, CT) to prepare a cortisol solution of 1 mg/mL. The cortisol solution was mixed with artificial saliva to concentrations of 0 (ethyl alcohol only), 3, 6, and 9 ng/mL. Concentrations of cortisol in saliva were selected to represent ranges of salivary cortisol concentrations reported for cattle challenged with ACTH (Negrão et al., 2004), or castration (González et al., 2010). Before the first experimental period, concentrations of salivary cortisol were verified by solid-phase RIA (Yates et al., 2010) using commercially available antibody-coated tubes (Seimens Diagnostics, Los Angeles, CA).

Collection Procedures and Sample Analysis

Samples were collected at 0, 3, 6, 9, 12, 24, 36, 48, 60 and 72 h for the 3 d treatment period and the 3 d post-treatment period. A syringe was used to collect 4 and 6 mL samples through a filtered suction strainer for analysis of VFA and NH_3, respectively. To prevent N volatilization, collection tubes were prepared with 1.25 mL of a 5% solution of 1 *M* HCl before sampling. Immediately after collection, ruminal fluid samples were centrifuged (Eppendorf North America) for 20 min at 20,000 × g and stored at -20°C until analysis. During each 3-d sampling period, liquid and solid effluent were collected into an insulated container on ice. Each morning, effluent volume was recorded, then homogenized for 1 min using a blender (Heavy-Duty BigStik; Waring Commercial, Torrington, CT), and a 500 g subsample collected and composited by fermentor (Lodge-Ivey et al., 2010). Daily composited samples were frozen at -20°C so that at the end of each 3-d collection period, approximately 1,500 mL had been collected from each fermentor. Artificial saliva (±10 mL) was collected from each fermentor daily, composited, and frozen (-20°C).

Ammonia was analyzed using the methods of Broderick and Kang (1980) adapted to a microtiter plate (BioTek Instruments, Winooski, VT). Concentrations of VFA were determined using gas chromatography (Agilent 7890, Agilent Technologies, Santa Clara, CA) according to the methods of Goetsch and Galyean (1983). Feed and effluent samples were analyzed for DM, OM, NDF, and CP to calculate digestibility. To determine DM, composited effluent was lyophilized (VirTis, Gardiner, NY) and a subsample was dried in a 105°C forced-air oven (845; Precision Scientific Group, Chicago, IL) for 24 h. Dry samples were ashed for 3 h at 500°C in a muffle furnace (F-A1730; Sybron Corp., Dubuque, IA) to determine OM. Samples were analyzed for NDF as described by Van Soest et al. (1991) using heat stable alpha-amylase. Sample CP concentrations were determined by measuring total N (Leco FP-528; Leco Corp., Henderson, NV) multiplied by 6.25.

Table 1. Ingredients and nutrient concentration of diet fed to rumen microbes in continuous flow fermentors

Item	% of DM
Corn grain, cracked	41.0
Sudan hay	15.0
Soybean hulls	20.0
DDGS[1]	15.0
Molasses	8.0
Urea	0.50
Limestone	0.30
Salt	0.20
Nutrient analysis[2], DM basis	
OM	92.7
NDF	31.4
CP	16.8

[1]DDGS = Dried distillers grains.
[2]Analyzed in the nutrition laboratory at New Mexico State University.

Statistical Analysis

Data were analyzed as a 4 × 4 Latin square using mixed models (SAS Inst. Inc., Cary, NC). Individual fermentors were the experimental unit. The statistical model for digestibility data included fermenter, period, and cortisol, and the model for fermentation end-products collected over time included fermentor, period, cortisol, hour, and cortisol × h. Compound symmetry was the covariance structure used for data with repeated measures within period. Contrasts included linear and quadratic effects of increasing cortisol concentrations of as well as no cortisol (0 ng/mL)

vs. the average of cortisol (3, 6, and 9 ng/mL). Treatment differences were considered significant when $P \leq 0.10$ and a tendency when $0.10 < P < 0.15$.

RESULTS

Fermentation Characteristics

Period of Cortisol Treatment. Cortisol × h ($P \leq 0.01$) were observed for acetate and valerate (Fig. 1); acetate was numerically greater for cortisol treatments (3, 6, and 9 ng/mL) than 0 ng/mL from 0 to 24 h, not different among treatments at 36 and 48 h, and numerically lower for 6 ng/mL than 0, 3, and 9 ng/mL at 60 and 72 h; valerate was not different among treatments from 0 to 12 h, but greater for 9 ng/mL than 0, 3, and 6 ng/mL at 24, 36, 48, 60, and 72 h. Ruminal NH_3 was not different ($P \geq 0.43$) among treatments. Total VFA tended to increase from 0 to 3 ng/mL, but were not different among 3, 6, and 9 ng/mL (quadratic, $P = 0.12$). Propionate was not different ($P \geq 0.49$) among cortisol treatments. Butyrate tended to decrease from 0 to 3 ng/mL cortisol, and was not different among 3, 6, and 9 ng/mL (quadratic, $P = 0.07$), whereas isobutyrate tended to increase from 0 to 3 ng/mL cortisol, and was not different among 3, 6, and 9 ng/mL (quadratic, $P = 0.13$). Isovalerate was not different among 0, 3, and 6 ng/mL, but decreased from 6 to 9 ng/mL of cortisol (quadratic, $P = 0.06$). Acetate-to-propionate ratio was not different ($P \geq 0.35$) among treatments.

Period after Cortisol Treatment. Isobutyrate was lower for 3, 6, and 9 than 0 ng/mL at 60 h (cortisol × h; $P = 0.03$; data not shown). Ruminal fluid pH was not different ($P \geq 0.27$) among treatments, but NH_3 decreased linearly ($P = 0.04$) with increasing cortisol. Total VFA, acetate, propionate, butyrate, and isobutyrate were not different ($P \geq 0.19$) among treatments. Valerate tended to increase linearly ($P = 0.14$) with increasing concentrations of cortisol. Isovalerate was lower for 9 ng/mL than 0, 3, and 6 ng/mL cortisol (quadratic, $P = 0.10$). Acetate-to-propionate ratio did not differ ($P \geq 0.49$) among treatments.

Digestibility

Period of Cortisol Treatment. Fermentors receiving cortisol at 9 ng/mL tended to have greater OM in effluent (OM output) than those that received 0, 3, and 6 ng/mL (quadratic, $P = 0.12$; Table 3). Similarly, NDF in effluent was greater for 9 ng/mL than 0, 3, and 6 ng/mL (quadratic, $P = 0.08$). As a result, digestibility of OM (g/d and % of intake) tended to be lower (quadratic, $P = 0.12$), and NDF digestion (g/d and % of intake) was lower (quadratic, $P = 0.09$) for 9 ng/mL cortisol than those that received 0, 3, or 6 ng/mL. Output and digestibility (g/d and % of intake) of CP were not different ($P \geq 0.23$) among treatments.

Period after Cortisol Treatment. Effluent (output) and digestibility (g/d and % of intake) of OM, NDF, and CP were not different ($P \geq 0.51$) among cortisol treatments (Table 3).

DISCUSSION

Previous research (Galyean et al., 1981; Fluharty et al., 1994; Gilliam et al., 2009) investigating the interaction between stress and rumen function in vivo has produced varying results. Discrepancies could be because some stressed animals, such as receiving calves, are subjected to both fasting and stress, which can produce different responses compared to stress alone (Loerch and Fluharty, 1999). The current study used an in vitro model to minimize the fasting effect of stressed animals.

Results suggest that exposure of rumen microbes 9 ng/mL cortisol impacts anaerobic fermentation and digestion. Since shifts in microbial populations were not quantified, we can speculate that decreases in NDF digestibility in response to 9 ng/mL cortisol suggest modifications of the bacterial population, and specifically, cellulolytic bacteria may have occurred. Ruminal fluid NH_3 concentrations were not affected by cortisol during the period of cortisol treatment, but were greater in the period after cortisol treatment for 3, 6, and 9 compared to 0 ng/mL cortisol, suggesting that bacteria may use N differently following exposure to cortisol.

As mentioned previously, differences in rumen fermentation and digestion associated with stress have been variable. Galyean et al. (1981) observed greater decreases in rumen bacteria and protozoa associated with fasting and transport than fasting alone. In contrast, Fluharty et al. (1994) reported no difference in fermentation, digestion of nutrients, and bacteria populations of stressed cattle 3 d after arrival at a feedlot compared to the day of arrival. However, the study of Fluharty et al. (1994) did not have a control group (no stress) of animals and therefore it is possible decreased DMI influenced their observed results. Gilliam et al. (2009) indicated that acetate concentrations were greater, and butyrate concentrations were lower in steers exposed to an endotoxin, which is consistent with the results of the current study. However, Gilliam et al. (2009) observed a tendency for greater NDF digestibility in stressed steers, which is in contrast to results of the present study. These discrepancies between studies could be a result of DMI and passage rate responses in the in vivo study (Gilliam et al., 2009), which were minimal in the current in vitro study. In addition to saliva, the enteric nervous system also supplies stress hormones to the gut (Verbrugghe et al., 2012) which may not only result in greater physiological concentrations of cortisol, but may also supply additional stress hormones, such as catecholamines. Catecholamines act as siderophones and auto inducers which results in increased growth and virulence factor production in bacteria (Hughes and Sperendio, 2008). Although the mechanisms by which glucocorticoids interact with bacteria have not yet been fully elucidated, steroid hormones are structurally similar to the quorum sensing molecule N-3-oxoocatnoyl-L-homoserine lactone and may influence biofilm production (Hughes and Sperendio, 2008). Therefore, stress hormones, either alone or in combination, could alter fermentation and digestibility patterns of ruminants.

IMPLICATIONS

The results of this study imply that rumen microbial fermentation and nutrient digestibility are altered by exposure to salivary cortisol concentrations of 9 ng/mL. Further research is warranted to determine how cortisol and other stress hormones relate to the physiological processes of gastrointestinal bacteria and animals that occur in vivo.

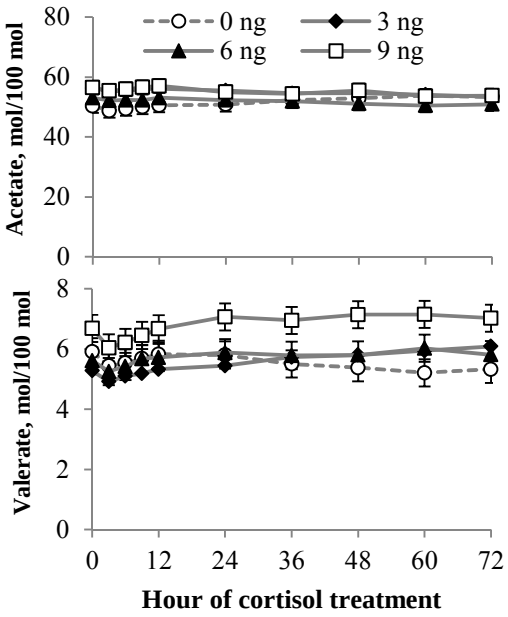

Figure 1. Acetate and valerate (mol/100 mol) of ruminal fluid in continuous flow fermentors infused (1.55 ± 0.05 mL/min) with artifical saliva containing 0, 3, 6, and 9 ng/mL cortisol. Effects for acetate were cortisol × h (P = 0.01), cortisol linear (P = 0.38), cortisol quadratic (P = 0.86), and no cortisol vs. cortisol (P = 0.27); Effects for valerate were cortisol × h (P < 0.01), cortisol linear (P = 0.09), cortisol quadratic (P = 0.22), and no cortisol vs.cortisol (P = 0.43).

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Table 2. Effects of cortisol concentrations in artificial saliva on fermentation characteristics of continuous culture fermentors

	Treatments[1]					Contrasts[2]			
	0	3	6	9	SEM	Cort × h	Lin.	Quad.	0 vs. Cort
Period of cortisol treatment									
NH_3, m*M*	2.20	1.64	2.00	1.91	0.36	0.71	0.76	0.53	0.43
Total VFA, m*M*	113	130	121	120	4.92	0.22	0.65	0.12	0.11
Individual VFA, mol/100 mol									
Acetate	51.2	55.1	52.0	55.2	2.10	0.01	0.38	0.86	0.27
Propionate	30.1	27.8	30.6	27.5	1.73	0.94	0.54	0.82	0.49
Butyrate	12.0	10.6	10.2	10.3	0.35	0.56	0.01	0.07	<0.01
Isobutyrate	0.03	0.07	0.05	0.04	0.02	0.69	0.88	0.13	0.24
Valerate	5.56	5.48	5.70	6.73	0.42	<0.01	0.09	0.22	0.43
Isovalerate	1.11	0.94	1.44	0.25	0.23	0.99	0.08	0.06	0.41
Acetate:propionate	1.80	2.15	1.74	2.18	0.20	0.69	0.42	0.83	0.35
Period after cortisol treatment									
NH_3, m*M*	2.64	1.63	2.09	1.28	0.31	0.64	0.04	0.76	0.03
Total VFA, m*M*	107	126	110	116	7.00	0.15	0.73	0.39	0.25
Individual VFA, mol/100 mol									
Acetate	52.1	52.4	50.9	54.3	2.63	0.96	0.67	0.57	0.88
Propionate	31.9	30.5	32.5	29.1	2.40	0.98	0.57	0.68	0.68
Butyrate	9.77	10.36	9.60	9.85	0.47	0.99	0.81	0.73	0.77
Isobutyrate	0.09	0.07	0.06	0.02	0.03	0.03	0.19	0.90	0.30
Valerate	5.33	5.96	5.64	6.63	0.47	0.66	0.14	0.71	0.22
Isovalerate	0.82	0.68	1.32	0.07	0.30	0.70	0.26	0.10	0.70
Acetate:propionate	1.69	1.90	1.63	2.07	0.27	0.98	0.49	0.69	0.58

[1] Treatments were cortisol concentrations (0, 3, 6, and 9 ng/mL) in artificial saliva infused (1.55 ± 0.05 mL/min) into fermentors.
[2] Cort. × h = cortisol × h interactions; Lin = linear effect of cortisol, Quad = quadratic effect of cortisol, 0 vs. Cort = contrast of no cortisol vs. cortisol.

Table 3. Effects of cortisol concentrations in artificial saliva on in vitro nutrient digestion in continuous culture fermentors

	Treatments[1]					Contrasts[2]		
	0	3	6	9	SEM	Lin.	Quad.	0 vs. Cort
Period of cortisol treatment								
Nutrient output[3], g								
OM	34.3	33.7	33.9	37.8	1.45	0.10	0.12	0.60
NDF	9.27	8.96	7.59	12.19	1.51	0.22	0.08	0.83
CP	12.21	12.09	12.49	12.77	0.35	0.23	0.58	0.58
Nutrient digested, g								
OM	43.1	43.7	43.5	39.6	1.75	0.10	0.12	0.60
NDF	11.66	11.97	13.34	8.75	1.50	0.22	0.08	0.83
CP	7.71	7.84	7.44	7.16	0.52	0.29	0.63	0.63
Digestibility, %								
OM	55.6	56.5	56.2	51.1	2.03	0.11	0.12	0.60
NDF	55.9	57.3	63.9	41.5	7.39	0.22	0.09	0.82
CP	38.5	39.4	37.2	35.9	2.18	0.29	0.58	0.67
Period after cortisol treatment								
Nutrient output[3], g								
OM	36.1	36.4	35.7	35.6	1.73	0.80	0.91	0.93
NDF	9.73	10.05	8.74	10.39	1.18	0.88	0.53	0.99
CP	12.83	12.88	12.69	12.78	0.57	0.89	0.97	0.94
Nutrient digested, g								
OM	41.4	41.1	41.7	41.7	2.01	0.84	0.92	0.95
NDF	11.07	10.85	11.99	10.34	1.19	0.82	0.51	0.99
CP	7.40	7.38	7.37	7.28	0.71	0.89	0.95	0.93
Digestibility, %								
OM	53.4	53.0	53.8	53.9	2.39	0.81	0.92	0.94
NDF	53.2	51.7	57.7	49.5	5.73	0.82	0.52	0.96
CP	36.4	36.2	36.7	36.1	3.20	0.97	0.94	0.98

[1] Treatments were cortisol concentrations (0, 3, 6, and 9 ng/mL) in artificial saliva infused (1.55 ± 0.05 mL/min) into fermentors.
[2] Lin = linear effect of cortisol, Quad = quadratic effect of cortisol, 0 vs. Cort = contrast of no cortisol vs. cortisol.
[3] Includes nutrients contained in the effluent collected from each fermentor.

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Shifting the paradigm of liver abscess dogma in USA feedlots[1]

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ABSTRACT: Liver abscesses in feedlot cattle are a major economic, welfare and production concern to the cattle feeding industry. Severe liver abscesses (**LA**) reduce ADG by as much as 0.20 kg, DMI by 5%, trimming loss by 0.43%, carcasses grading choice by 7%, and HCW by 36 kg. In processing facilities, LA introduce operational and food safety concerns. These include a reduction in processing efficiency, lost time as a result of line stoppages and offal condemnation in addition to the consumer risk associated with LA contamination of edible meat. Tylosin phosphate, a macrolide antibiotic, has been shown to reduce LA by 75% and level of *Fusobacterium Necrophorum* in the rumen by 80 to 90%. During this initial observational study, a total of 83 feedlot pens (each individual feed yard exceeding 40,000 head capacity) within three geographical regions (Arizona, Colorado and the Texas Panhandle) were sampled. Feedlot pen data were collected within 1 wk prior to harvest and cattle were traced to the packing plant. Every third rumen and its matching liver were tagged (if condemned only). Rumens were scored for consolidation, scars, moderate and acute lesions and a sample was taken. Livers were scored based on an adaptation of the Elanco Liver Check scoring system. Holstein cattle had a greater ($P < 0.05$) percentage of LA than beef breeds (30.3 vs. 20.0%). Additionally, Holstein cattle had 11% severe LA (A+) compared with 4% for beef breeds ($P < 0.05$). No geographical difference ($P \geq 0.10$) were detected for liver abscess prevalence and averaged 23, 25 and 26% for The Texas Panhandle, Arizona and Colorado region respectively. Liver abscess rate and severe LA (A^+) incidence differed between feedlots ($P < 0.05$) with within feedlot variation. A correlation was observed for LA% and days on feed (R^2 =0.22; $P = 0.04$) and for LA % and breed (R^2= 0.29; $P = 0.01$). No correlation was observed between LA percentage and tylosin phosphate, and between LA percentage and rumen lesions ($P \geq 0.10$). This data indicated no association between LA and rumen damage as a result of acidosis. Rumen lesions averaged 12.2%, of which 9.3% were consolidated, 2.4% scar tissue, and the remainder moderate and acute lesions. This study justifies further investigation of feedlot soil and manure as the source of LA causing pathogens to evaluate the within feedlot variation observed for in LA percentage in cattle.

Key words: cattle, feedlot, liver abscess

INTRODUCTION

Liver abscesses in feedlot cattle are a major economic, welfare, and production concern. Severe liver abscesses (**LA**) reduce ADG between 0.06 kg to 0.2 kg (Rezac et al., 2014), DMI by 5% (Brink et al., 1990), trimming loss by 0.43% (Montgomery et al., 1985), choice grade by 7% (Fox et al., 2009), and HCW by 4 kg to 36 kg (Montgomery et al., 1985; Fox et al., 2009; Rezac et al., 2014). Tylosin phosphate, a macrolide antibiotic, has been shown to reduce LA by 75% (Nagaraja et al., 1996) and the level of *Fusobacterium Necrophorum* in the rumen 80 to 90% (Nagaraja et al., 1999b). The theory of liver abscess formation dictates *F. Necrophorum*, an opportunistic anaerobic bacterium and common inhabitant of the rumen, colonizes rumen lesions caused by feeding high concentrate diets resulting in low pH levels for extended periods of time. Bacterial entry via these ulcerated cites into the portal blood system then results in liver abscesses (Nagaraja and Chengappa, 1998). Tylosin phosphate is included in 77% of all commercial feedlot diets (USDA, 2011) to control LA. However, even in the presence of tylosin phosphate, since 2003 there has been a slight increase in LA prevalence in beef breeds from 12% in 2003 to 16% in 2013 and a linear increase in Holstein cattle from 12% in 2003 to 55% in 2013 (Elanco, 2014; Reinhardt and Hubbert, 2015). In light of the Veterinary Feed Directive, the metaphylactic use of antibiotics will be strictly controlled to monitor risks associated with antimicrobial resistance. We hypothesized there were no correlations between LA and antibiotic growth promotor usage nor between LA and rumen damage as a result of rumen acidosis. The objectives were to investigate the correlation between LA and days on feed (**DOF**), location, climate, breed type, gender, stocking density, and use of an antimicrobial and the presence of rumen lesions. Within yard, pen to pen LA prevalence variation were also determined.

MATERIALS AND METHODS

General

Approval from the New Mexico State University Institutional Animal Care and Use Committee was not required as procedures used in this study were confined to commercially harvested carcasses, records were retrospectively evaluated and no procedure was performed on live animals. Fourteen commercial feed yards (larger than 40,000 head each) and three commercial packing plants were surveyed from three geographical regions: Arizona, Colorado and the Texas Panhandle. All cattle were

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grown in the summer months and harvested between 28th August 2015 and 20th November 2015 representative of the summer growing period. The first step in planning the survey involved communicating with the packing plant and feed yards for show lists and harvesting schedules. Pens of cattle were then selected to be slaughtered over an entire week at each packing plant including both an A and B shift where applicable. Line speeds differed between plants averaging between 6 to 8 s per carcass. Cattle slaughtered at all three plants were transported no more than 483 km. All cattle were slaughtered on day of arrival thus no adjustment for DOF were deemed necessary. The survey team consisted of graduate students in animal science.

Gross Pathology Scoring and Sampling

Lot, pen, and drive (lots subdivided into manageable sizes of between 25 to 200 head depending on the plant) information and animal pen numbers were cross referenced to ensure proper identification of selected pens. A student was positioned at the start of the offal line to identify carcass numbers and signal the start of each drive to the rest of the team. To link individual animal livers with rumen scores, livers and rumens were tagged using a USDA approved tagging gun and colored, numbered tags at the evisceration table. Students consecutively tagged every third rumen (irrespective of condemnation status of the liver) within a drive whilst a second student tagged the corresponding liver (only if condemned). Condemned livers were removed from the end of the offal line and channeled to a separate working station in the rendering area. Students individually assessed each condemned, labelled liver within the selected drives. A photo was taken from each liver and the number and color of the tag recorded. Hepatic abscesses were scored using a modified Elanco Liver Check System (Elanco, Greenfield, IN). Livers, free from abscesses or scars were scored **N** (normal), livers displaying less than 2 abscesses of less than 2 cm in diameter were scored **A**$^{-}$, livers with 2 to 4 abscesses 2 to 4 cm in diameter were scored **A** and livers with more than 1 abscess larger than 4 cm or more than 4 abscesses larger than 2 cm were scored **A**$^{+}$. Abscessed livers adhered to the body cavity or diaphragm received an additional score, '**D'** and ruptured abscesses were scored '**R'**. A student was positioned at the offal line recording the number of condemned livers within each drive to account for livers condemned but sent to rendering as a result of contamination. This allowed the research team to adjust condemnation rate to prevent an underestimation of LA prevalence. Livers were scored and sampled for abscesses by collecting all condemned livers after the offal table. All rumens were retrieved except those condemned and sent to rendering. Rumens were washed and hung and two team members investigated each rumen. The tag number and color were recorded and the rumen was investigated for lesions. Scores were assigned using an adapted scoring system described by Thomson et al. (1967) and Rezac et al. (2014).

Statistical Analysis

Associations between LA and DOF, location, climate, breed type, gender, stocking density, antimicrobial use, and rumen lesions were analyzed using Pearson's correlation of SAS (SAS Inst. Inc., Cary, NC). Differences with $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

There were no differences in LA % across the three regions surveyed ($P \geq 0.10$), averaging 23, 25 and 26% for the Texas panhandle, Arizona and Colorado, respectively. Feed yards and pens sampled within the three regions all contained Holstein and beef breeds and both steers and heifers. A breed effect was detected for LA % (R^2= 0.29; P = 0.01) but no correlation ($P \geq 0.1$) between LA % and gender (Table 1). As represented in Fig. 1, DOF significantly correlates with LA % (R^2 =0.22; P = 0.04) as has been previously observed (Elanco, 2014). Weather data were collected from weather stations in close proximity to each feed yard and no correlations were observed between LA % and maximum and minimum temperatures or level of precipitation as measured across the feeding period of cattle surveyed ($P \geq 0.10$). Stocking density had no effect on LA % nor did the in and out weights of cattle surveyed ($P \geq 0.10$). Feed yards and pens surveyed in this study included a significant proportion of naturally fed cattle (no tylosin phosphate or ractopamine hydrochloride) and no correlations were detected between LA % and the use of tylosin phosphate ($P \geq 0.10$). These results are consistent with the increasing trend in LA % seen in both beef and Holstein cattle fed tylosin phosphate (Elanco, 2014). Comparing the distribution of LA between beef breeds and Holstein cattle (Fig. 1), beef breeds averaged 20% LA and Holsteins 30%. Severe LA (A+) were 4 and 11% for beef and Holstein cattle, respectively ($P < 0.05$).The LA % for beef cattle correspond to the 12 to 18% value reported by Elanco (2014). Livers affected by flukes, cirrhosis and other abnormalities were also assigned to the normal group for abscess investigation. A final reason for different prevalence data could be that rumens were tagged and collected and used to establish the sampling frequency of livers within a lot or drive in the packing plant. Therefore, abscess data collected represent each lot negating any effects of commercial packing plant line speeds on sampling frequency. Adhesions and ruptured abscesses between Holsteins and beef cattle (9 vs. 2 %) were different ($P < 0.05$), resulting in regular line stoppages for disinfection. Rumen damage % (as measured by the amount of consolidation, scarring and moderately and acutely infected lesions) varied from 5.3 to 24% between yards (Table 2). Rumen damage was only measured for the Colorado and Arizona regions and averaged 14 and 10% respectively ($P \geq 0.10$). In a survey conducted by Rezac et al. (2014) 22.5% mild rumen lesions and 9.8% severe lesions were reported. The mild classification as applied by Rezac et al. (2014) is comparable to the consolidated, scarring and moderate lesion categories in this study, appearing much lower. The size of the current dataset and the unbiased selection of yards across the three locations,

including both naturally fed and conventionally fed cattle as well as allowing for Holstein, Colored cattle and the gender differentiation might contribute to a more balanced representation of the current level of rumen damage in commercial feed yards. Significant between yard variations in rumen damage were detected; however no correlation were detected between LA % and rumen damage ($P \geq 0.1$).

Results obtained from this study suggest no association between liver abscess incidence and the level of rumen damage occurring as a result of acidosis. This challenges the rumenitis hepatic abscess theory as proposed by Nagaraja and Chengappa (1998). No association between LA % and the use of tylosin phosphate could be detected. This corresponds with the linear increase in LA % observed in Holstein cattle over the last 10 years (Elanco, 2014).

CONCLUSIONS

Data collected from this study suggests breed and DOF affect LA. Significant variation in LA % within feed yards and severity of LA were detected suggesting factors within feed yard impact the development of LA. An alternative hepatic entry route for LA causing pathogens needs to be investigated in lieu of the hepatic abscess rumenitis complex theory.

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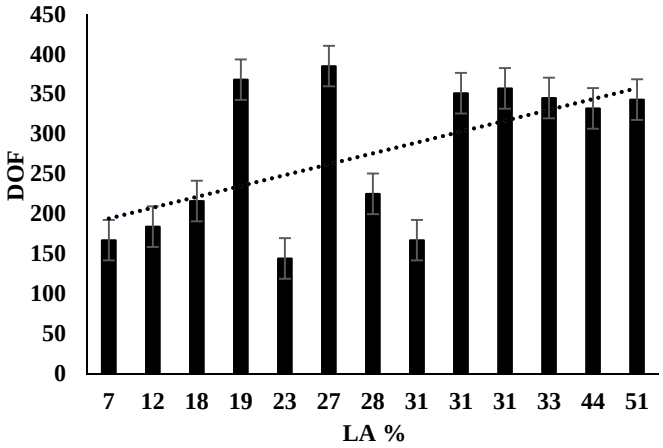

Figure 1. The correlation between liver abscess % (LA) and days on feed (DOF; R^2 =0.22, P = 0.04) for thirteen different feed yards across three locations, Arizona, Texas Panhandle and CO.

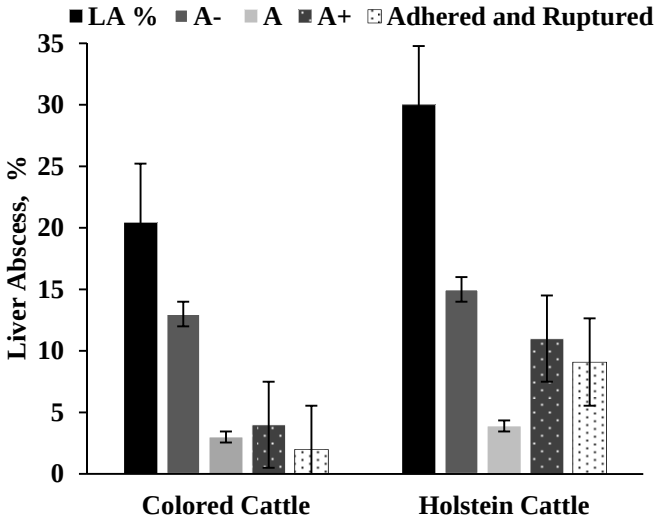

Figure 2. Difference in liver abscesses (LA) prevalence between Colored and Holstein cattle across different regions: Arizona, Texas Panhandle and Colorado. Total LA % were determined and abscesses classified into A^- (< 2 abscesses, < 2 cm diameter); A (2 to 4 abscesses, 2 to 4 cm in diameter) and A^+ (> 1 abscess larger than 4 cm; > 4 abscesses larger than 2 cm). An additional score were assigned fir abscesses adhered to body wall and or diaphragm. Correlation between LA % and breed (R^2= 0.29; P = 0.01).

Table 1. Correlation of various parameters with liver abscess percentage.

	Area	Yard	Pen	Max Temp °C	Low Temp °C	Precip mm	Stocking Density Sqft/hd	Breed	Sex	In Weight kg	Out Weight kg	DOF	Tylan	Rumen Lesions %
r^2	0.04	-0.01	-0.09	-0.07	-0.12	-0.01	-0.07	0.29	0.17	-0.17	0.02	0.22	-0.13	-0.11
P-value	0.72	0.91	0.44	0.55	0.29	0.91	0.51	0.01	0.12	0.13	0.87	0.04	0.26	0.37
n	83	83	83	83	83	83	80	83	83	80	78	80	80	63

Table 2. Distribution of liver abscess and rumen lesion scores between feed yards.

	Feed Yard[1]														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	SEM
Liver Abscess %	14.2	8.3	32.8	12.0	50.5	39.3	33.9	9.5	15.5	25.2	28.6	19.3	21.2	21.3	1.81
A^-	11.4	6.0	16.4	12.0	23.5	24.7	18.1	9.5	11.2	14.6	15.4	13.7	9.8	11.0	0.90
A	0.6	0.5	2.0	0.0	5.0	0.0	6.1	0.0	3.5	3.9	4.1	0.0	5.1	6.0	0.48
A^+	2.0	2.0	13.8	0.0	22.5	14.3	9.9	0.0	1.2	6.9	8.9	5.7	6.2	4.3	1.05
Rumen Lesion %							5.8	24.0	12.2	10.9	17.3	6.3	16.1	5.3	2.51
Consolidation							3.4	17.0	8.0	8.4	14.5	5.7	13.1	4.7	1.12
Scars							2.1	6.0	2.8	1.3	2.3	0.7	3.2	0.7	0.39
Moderate Lesions							0.2	1.0	1.7	0.7	0.4	0.0	0.1	0.0	0.18
Acute Lesions							0.2	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.09

[1]Feed Yards sampled all exceeded 40,000 head capacity.

YOUNG SCHOLARS

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Altered rumen microbial populations in response to high sulfate water in lambs

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ABSTRACT: High levels of dietary sulfur (S) and S-compounds can be detrimental to livestock health and performance. Research in this area has focused on ruminant species because of the potential for H_2S buildup in the rumen caused by ruminal bacteria. However, despite an abundance of research, there has been little gain in development of effective treatment and prevention strategies for ruminant livestock in high S environments. This may be due to the complex interaction between the rumen microbiome and the host animal. Research in our laboratory has focused on high sulfate (SO_4^{2-}) drinking water because of its prevalence in the Western U.S. The focus of the present research was to determine the impact of high SO_4^{2-} drinking water on the rumen microbiome of growing lambs. We conducted two trials: an initial trial (Trial 1) to establish changes in the rumen microbiome associated with high SO_4^{2-} water, and a follow-up trial (Trial 2) to confirm rumen microbial species involved in the response to high SO_4^{2-} and also identify specific microbial species capable of adapting to a SO_4^{2-} challenge. Each trial consisted of individually penned Hampshire-cross lambs (n = 43 in Trial 1; n = 16 in Trial 2) which had access to *ad libitum* feed and high SO_4^{2-} water (3,000 mg SO_4^{2-}/L) for a 28 d period. DNA was extracted and sequenced from d 0, 7, and 28 rumen samples from 8 lambs per trial and compared with known 16S rDNA reads for microbial identification. Operational taxonomic units (OTU) were defined as sequence clusters with ≥ 97% identity and analyzed for the fixed effect of sampling day using the GENMOD procedure of SAS. Volatile fatty acid (VFA) concentrations were also determined and analyzed for sampling day effect using the GLM procedure. DNA results for Trial 1 generated a total of 145 OTU found in at least 1 of the 24 sequenced samples; 8 OTU were affected ($P \leq 0.05$) by sampling day. Trial 2 resulted in 287 OTU identified in at least 1 of the 24 sequenced samples, with sampling day affecting ($P \leq 0.05$) 38 of those OTU. Isovalerate was affected ($P = 0.01$) by sampling day in Trial 1. There was a linear increase in acetate ($P = 0.029$) and linear decreases ($P \leq 0.055$) in valerate and proprionate concentrations in Trial 2. Collectively, our results indicate a shift in rumen microbe relative abundance in response to high SO_4^{2-} water. Abundance variation may explain differences in host animal ability to tolerate and adapt to high SO_4^{2-}. Similarities in microbial abundance changes across the two trials suggest that particular species are especially reactive to high ruminal SO_4^{2-} and are likely important to host response. Furthermore, certain microbial species demonstrated greater potential to adapt over time to a high SO_4^{2-} environment. Greater understanding of the rumen microbes involved in the response to high SO_4^{2-} is necessary for development of effective treatment and prevention strategies for ruminant livestock maintained in high SO_4^{2-} water regions.

Key words: DNA sequencing, microbes, rumen, sulfate, volatile fatty acids

INTRODUCTION

In the western U.S., livestock frequently only have access to water sources that are less than ideal due to competition with urbanization and mineral extraction (Raisbeck et al., 2007). Livestock drinking water is often high in sulfur (**S**), especially in the form of sulfate (**SO_4^{2-}**). Ruminants possess the unique ability to reduce dietary S and SO_4^{2-} via microbes within the rumen, and therefore drinking water sources high in either of these can greatly impact dietary S intake values (Nichols et al., 2012). Sulfur-reducing bacteria (e.g., *Desulfovibrio* and *Desulfotomaculum*) can utilize lactate and SO_4^{2-} as substrates to generate acetate and S^{2-}, which can further react with free H^+ to form H_2S which then accumulates in the rumen gas cap. The gas can be eructated and re-inhaled by the animal, causing neural damage and the onset of S-induced polioencephalomalacia (Gould et al., 2002). It has been proposed that the bacteria responsible for SO_4^{2-} reduction are capable of adapting to changing levels of dietary S and SO_4^{2-} (Cummings et al., 1995a). Variation in host animal tolerance to high dietary S has been demonstrated in beef cattle (Cammack et al., 2010). This variation is postulated to be partially due to differences in ruminal SO_4^{2-} reducing bacteria populations which are capable of adapting to changing levels of dietary S. Furthermore, volatile fatty acids (**VFA**) are a product of microbial fermentation and typically provide 50-80% of metabolizable energy for the host ruminant (Church, 1988). Zinn et al. (1997) demonstrated altered VFA production in response to high S intake. Utilization of S by specific microbial species, as well as indirect effects stemming from reduced feed intake and an altered rumen environment, have been associated with modified rates and products of microbial fermentation and the subsequent absorption of end products in the rumen (Dijkstra et al., 1993). As such, shifts in rumen microbial populations and abundance resulting from high S intake may impact the production of VFA and energy availability to the host ruminant. Therefore, we hypothesized that the rumen microbiome and subsequent

VFA production will be altered in response to a high SO_4^{2-} drinking water treatment in growing lambs. Our objectives were to determine and confirm changes in abundance of rumen microbial species and identify microbial species that potentially adapt to a high SO_4^{2-} environment. Furthermore, we sought to establish shifts in ruminal VFA concentrations stemming from high SO_4^{2-} derived rumen microbiome changes. A better understanding of the relationship between dietary SO_4^{2-} intake and individual rumen microbial species could facilitate the development of viable prevention and treatment methods to optimize livestock health and performance in regions with high SO_4^{2-} water.

MATERIALS AND METHODS

Animal Care. All procedures were approved by the University of Wyoming Animal Care and Use Committee. The initial lamb trial (Trial 1) occurred July 27th to August 23rd, 2013, at the University of Wyoming's Laramie Research Extension Center. The follow up trial (Trial 2) took place February 19th to March 26th, 2015, at the same location. Hampshire-cross growing lambs (n = 43; with initial BW 48.76 ± 16.44 kg in Trial 1; n = 12; with initial BW 75.52 ± 14.4kg in Trial 2) were randomly allotted to individual pens in a confinement facility for a 28 d feed and water intake trial. Trial 2 also included a 7 d post-treatment period to allow for collection of recovery data to be analyzed at a later time. Lambs were acclimated to the pelleted forage diet for 23 d prior to the start of Trial 1 and 20 d prior to Trial 2 to allow for adjustment to the diet as well as individual pens. All animals were administered the same forage-based pelleted diet (67.7% alfalfa and 27.5% wheat middlings; 16.2% CP, 36.3% NDF, 2.31 Mcal ME/kg, DM basis). Lambs were fed *ad libitum*, and orts were weighed back daily. Rumen samples were collected on d 0, 7, and 28 from all lambs along with 2-d averaged BW.

Water Sulfate. Prior to the high SO_4^{2-} water administration, lambs were provided water from the research facility with 67 mg SO_4^{2-}/L. To create the desired level of 3,000 mg SO_4^{2-}/L for the high S water treatment, sodium sulfate (Na_2SO_4) was mixed daily with water from the research facility. The water mixture was tested daily during the first 2 wk of SO_4^{2-} administration at the Wyoming Department of Agriculture Analytical Services (Laramie), and then every other day for the remainder of the treatment period. Actual levels over the duration of the trial period averaged 3043.89 ± 746.11 mg SO_4^{2-} /L during Trial 1 and 3,000 ± 500 mg SO_4^{2-}/L for Trial 2. This level of SO_4^{2-} was chosen to avoid acute toxicity. Water was provided *ad libitum*; disappearance was measured twice daily to determine lamb water intake. Water evaporation was also monitored daily.

Animal Selection. For each trial, 8 lambs were selected for DNA sequencing based on individual health and performance data, including feed and water intake. No signs of sPEM were observed in any of the lambs.

Microbial DNA Sequencing. From the rumen fluid samples, DNA was extracted using methods detailed by Yu and Morrison (2004); 5 µg of 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 by truncating each read after the first run of 3 bases using a phred quality score < 15, quality-trimmed by omitting reads with < 85 base pairs or a quality score of < 25, and compared with a database of 27K known 16S rDNA genes using the Bowtie reference-based assembly tool (Johns Hopkins, Baltimore, MD). For the read sequence and the database sequence to match, they were required to have ≥ 97% likeness. Operational taxonomic units (OTU) were defined as sequence clusters with ≥ 97% identity; therefore, each OTU was assumed to be a single microbial species.

Volatile Fatty Acids. Rumen samples from the selected lambs (24 samples in each Trials 1 and 2) were filtered and centrifuged to separate the supernatant from any particles present. Metaphosphoric-2 ethyl butyric acid (25%) was added at a 5:1 ratio. The mixture was incubated on ice, centrifuged for 15 min, and the supernatant transferred to a vial for analysis using gas chromatography with the 6980n Network GC system (Agilent, Santa Clara, CA) for determination of VFA concentrations.

Statistical Analysis. The OTU data sets from each of the two trials were analyzed separately, to confirm results from the initial trial. A generalized linear model was fitted using the GENMOD procedure of SAS to determine the effects of sampling day on OTU abundance assuming a Poisson distribution and using total OTU counts as a covariate; $\alpha = 0.05$ was considered statistically significant. Raw *P*-values from the Poisson regression were corrected for multiple tests using the false-discovery rate correction of Benjamini and Hochberg (1995). Because means produced from the Poisson regression were non-normally distributed, treatment means were generated using the GLM procedure of SAS. Additionally, those OTU with a significant sampling day effect were further tested for linear and quadratic effects using orthogonal contrasts. Finally, the GLM procedure was used to determine effects of sampling day (d 0, 7, or 28) on VFA molar percentages. Post hoc analysis comparing treatment means was conducted with the LSMEANS procedure of SAS using a Tukey's adjustment and assuming $\alpha = 0.05$.

RESULTS AND DISCUSSION

Trial 1 resulted in a total of 145 OTU found in at least 1 of the 24 sequenced samples (8 lambs; 3 sampling dates). Taxa with ≤ 3 counts (n = 42) for any one sample were considered to be sequencing artifacts (or potentially false positives) and thus eliminated. Of the remaining taxa (n = 103), 8 OTU were affected ($P \leq 0.01$) by sampling day. Trial 2 resulted in 287 OTU identified in at least 1 of the 24 sequenced samples. After removal of sequencing artifacts, 167 OTU remained and sampling day affected ($P \leq 0.05$) the abundance of 23 of those remaining OTU (Table 2). There were five microbial species that were consistently affected ($P \leq 0.05$) by sampling day across the two trials: *Prevotella ruminicola, P. albensis, P. nigrescen, P. genomosp.,* and *Butyrivibrio fibrisolvens.*

In addition to being the primary genus affected by sampling day, *Prevotella* comprised the largest portion of the microbiome in all lambs throughout both trials. It has been well established that *Prevotella* is typically the most dominant genus in the rumen (Stevenson and Weimer, 2009). Of the individual *Prevotella* species, *P. ruminicola* had the greatest abundance across trials regardless of sampling day. A linear increase ($P = 0.004$) in abundance of *P. ruminicola* was observed over the course of Trial 1; however, a linear decrease ($P \leq 0.001$) in *P. ruminicola* abundance was observed during Trial 2. Van Soest (1994) defined *P. ruminicola* as a carbohydrate fermenter that is able to utilize cellulose, hemicellulose, and pectin as well as starches, proteins, and sugars (both simple and complex). As this species can utilize a wide variety of substrates, it tends to be a predominant species within the rumen (Carberry et al., 2012). *Prevotella albensis* abundance increased ($P = 0.004$) initially at d 7 during Trial 1, but did not increase ($P = 0.015$) until d 28 in Trial 2. There was a quadratic effect ($P \leq 0.004$ in Trial 1; $P = 0.014$ in Trial 2) observed in abundance of *P. nigrescen* during both Trials 1 and 2. The return to pre-treatment abundance levels by d 28 suggests that *P. nigrescen* may have the ability to adapt to a high SO_4^{2-} environment. Finally, an unknown *Prevotella* species, *P. genomosp.*, was affected ($P \leq 0.001$) by sampling day in both trials. However, while it appears that these species are responsive to a high SO_4^{2-} environment, it is unclear if these are indeed the same species of *Prevotella*.

Butyrivibrio fibrisolvens decreased linearly ($P = 0.001$ in Trial 1; $P = 0.020$ in Trial 2) over the two trial periods, to nearly non-present by d 28. While *B. fibrisolvens* can utilize a wide variety of substrates, it is primarily noted for its ability to degrade cellulose and produce butyrate as the major fermentation acid (Hespell et al., 1993). Altered abundance of fibrolytic bacteria, including *B. fibrisolvens*, has been associated with S supplementation (McSweeny and Denman, 2007). While *B. fibrisolvens* can be cultured, the capacity of this species to persist and maintain abundance in the rumen will require further research (Klieve et al., 2003).

No differences in S-reducing bacteria were apparent in this study. Cummings et al. (1995b) reported an increase in H_2S production but not in S-reducing bacteria in response to S supplementation. This supports our lack of abundance changes in S-reducing bacteria, suggesting that S-reducing bacteria may respond to high SO_4^{2-} through increased enzymatic activity rather than a significant shift in population abundance. Conversely, fiber utilizing rumen microbial species have been shown to react to SO_4^{2-} with notable shifts in abundance (Morrison et al., 1990).

There was an effect ($P < 0.01$) of sampling day on isovalerate concentration in Trial 1, with the greatest molar percentage occurring on d 7 and the lowest on d 28 (Table 3). No other VFA concentrations were affected by sampling day in Trial 1. Uwituze et al. (2011) reported that steers on a high-S diet had greater 3-methyl isovalerate concentrations than steers on a low-S diet, suggesting that isovalerate production is susceptible to high dietary S. In Trial 2, there was a linear increase in acetate ($P = 0.029$) and a linear decrease ($P = 0.027$) in valerate molar proportions. Additionally, propionate tended to decrease linearly ($P = 0.055$) over the trial period (Table 4). Similar to our results, an increase in acetate production in response to a high S diet was reported by Uwituze et al. (2011). Additionally, Richter (2011) reported steers fed a high S diet for 36 d had lower valerate concentrations than steers on a control, low S diet; however, this reduction in valerate was not maintained over the course of a 155 d trial.

A lack of congruency is evident across many S-related studies. There are many factors that influence dietary S requirements, such as diet composition, metabolic status, livestock species and breed type, as well as rumen microbial fermentation and protein synthesis. Additionally, microbial and host response to SO_4^{2-} has been shown to vary. It is likely that these factors were contributing elements for variation observed between the two trials presented here. While the lambs in these two trials were administered the same diet and similar high SO_4^{2-} water, the trials were conducted in different seasons, which may have influenced water intake (e.g., greater water intake during Trial 1 versus Trial 2). While there were some differences in population abundance patterns, the reoccurrence of specific species across trials suggest that these microbes are particularly responsive to a high SO_4^{2-} environment. Changes in various microbial species may be driving shifts in VFA production. Furthermore, alterations in abundance or the adaptation of certain *Prevotella and Butyrivibrio* species may influence individual host animal ability to tolerate high SO_4^{2-} drinking water. Although no differences for S-reducing bacteria were apparent in the DNA sequencing data, the microbial differences that were observed could lead to a greater understanding of the relationship between the rumen microbiome and the host animal response to high levels of SO_4^{2-}.

IMPLICATIONS

Because rumen microbes are responsible for the degradation of consumed feedstuffs, ruminant livestock possess the unique ability to reduce dietary SO_4^{2-}. As a result, ruminant livestock are also subject to health complications associated with the overproduction of H_2S by the SO_4^{2-} reducing bacteria. Results from the current study demonstrate that there is a shift in rumen microbe abundance in response to high SO_4^{2-} water, and also indicate that certain microbial species may be particularly responsive to SO_4^{2-} and play an important role in host response. A greater understanding of the impact of high dietary S on resulting VFA production and absorption is also necessary to negate the effects on energetic efficiency and performance in the host ruminant. Because ruminant livestock in the western U.S. frequently encounter high SO_4^{2-} drinking water sources, it is imperative that feasible and effective treatment and management strategies be developed for affected livestock. A better understanding

of the role of the rumen microbiome in host response to high dietary SO_4^{2-} is critical to this endeavor.

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Table 1. Abundance least-squares means[1] of taxa significant for sampling day effect in lambs administered high SO_4 water for a 28 d trial period (Trial 1).

	Sampling Day			
Taxa	d 0	d 7	d 28	*P*-values
Prevotella ruminicola	125.85[a]	166.96[b]	178.69[b]	<0.001
Prevotella genomospecies	8.75[a]	13.98[b]	13.52[b]	0.001
Prevotella albensis	3.05[a]	5.04[b]	5.66[b]	0.004
Prevotella nigrescens	1.51[ab]	2.33[a]	0.53[b]	0.004
Butyrivibrio fibrisolvens	2.08[a]	1.01[ab]	0.16[b]	0.001

[1]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.

[a,b]Least squares means without a common superscript differ ($P < 0.05$).

Table 2. Abundance least-squares means[1] of taxa significant for sampling day effect in lambs administered high SO_4 water for a 28 d trial period (Trial 2).

	Sampling Day			
Taxa	d 0	d 7	d 28	*P*-values
Prevotella ruminicola	141.69[a]	111.54[b]	110.38[b]	<0.001
Prevotella genomospecies	1.57[a]	0.55[ab]	0.21[b]	0.001
Prevotella genomospecies	6.41[a]	2.59[b]	3.51[b]	<0.001
Prevotella albensis	4.70[a]	7.30[ab]	8.89[b]	0.015
Prevotella nigrescens	0.33[a]	0.17[a]	0.99[a]	0.014
Butyrivibrio fibrisolvens	1.08[a]	1.17[a]	0.241[a]	0.020

[1]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.

[a,b]Least squares means without a common superscript differ ($P < 0.05$).

Table 3. Least squares mean volatile fatty acid (VFA) molar percentages in lambs administered high SO_4 water for a 28 d trial period (Trial 1).

	Sampling Day				*P*-values
VFA	0	7	28	SEM	Day
Acetate	62.1	61.6	62.9	0.71	0.438
Propionate	20.0	19.3	19.2	0.57	0.538
Isobutyrate	0.9[x,y]	1.0[x]	0.7[y]	0.08	0.070
Butyrate	14.8	15.9	15.3	0.85	0.647
Isovalerate	0.8[x]	0.9[x,y]	0.6[z]	0.09	0.075
Valerate	1.4	1.3	1.3	0.07	0.630

[a,b,c]Least squares means within a row with different superscripts differ ($P < 0.05$).

[x,y,z]Least squares means within a row with different superscripts tend to differ ($P < 0.10$).

Table 4. Least squares mean volatile fatty acid (VFA) molar percentages effect in lambs administered high SO_4 water for a 28 d trial period (Trial 2).

	Sampling Day			*P*-values	
VFA	0	7	28	SEM	Day
Acetate	68.1	69.5	69.5	0.004	**0.029**
Propionate	16.5	15.0	14.6	0.005	0.055
Isobutyrate	0.8	0.7	0.6	0.0001	0.145
Butyrate	12.6	13.0	13.5	0.004	0.173
Isovalerate	0.7	0.6	0.5	0.0001	0.122
Valerate	1.3	1.1	1.2	0.0004	**0.027**

[a,b,c]Least squares means within a row with different superscripts differ ($P < 0.05$).
[x,y,z]Least squares means within a row with different superscripts tend to differ ($P < 0.10$).

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Effects of Organic or Inorganic Co, Cu, Mn, and Zn Supplementation to Late-Gestating Beef Cows on Productive and Physiological Responses of the Offspring

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ABSTRACT: Eighty-four multiparous, non-lactating, pregnant Angus × Hereford cows were ranked by pregnancy type (AI = 56, natural service = 28), BW, and BCS, and allocated to 21 drylot pens at the end of their 2nd trimester of gestation (d 0). Pens were assigned to receive forage-based diets containing: 1) sulfate sources of Cu, Co, Mn, and Zn (INR), 2) an organic complexed source of Cu, Mn, Co, and Zn (AAC; Availa®4; Zinpro Corporation, Eden Prairie, MN), or 3) no supplemental Cu, Co, Mn, and Zn (CON). Diets were offered from d 0 until calving, and formulated to meet requirements for energy, protein, macrominerals, Se, I, and vitamins. The INR and AAC diets provided the same daily amount of Cu, Co, Mn, and Zn. Cow BW and BCS were recorded, and liver samples were collected on d -10 and 2 wk (d 75) before the calving season. Within 3 h after calving, calf BW was recorded, liver samples were collected, and the expelled placenta was retrieved (n = 47 placentas). Calves were weaned on d 283 of the experiment, preconditioned for 45 d (d 283 to 328), transferred to a growing lot on d 328, and moved to a finishing lot on d 440 where they remained until slaughter. Liver Co, Cu, and Zn concentrations on d 75 were greater ($P \leq 0.05$) for INR and AAC compared with CON cows, whereas INR had reduced ($P = 0.04$) liver Co but greater ($P = 0.03$) liver Cu compared with AAC cows. In placental cotyledons, Co concentrations were greater ($P \leq 0.05$) in AAC and INR compared with CON cows, whereas Cu concentrations were only increased ($P = 0.05$) in AAC compared with CON cows. Calves from INR and AAC had greater ($P < 0.01$) liver Co concentrations at birth compared with calves from CON cows. Liver Cu and Zn concentrations at birth were greater ($P \leq 0.05$) in calves from AAC compared with cohorts from CON cows. Weaning BW was greater ($P \leq 0.05$) in calves from AAC compared with cohorts from CON cows, and this difference was maintained until slaughter. In the growing lot, calves from AAC cows had reduced ($P < 0.01$) incidence of bovine respiratory disease compared with CON and INR cohorts. Collectively, these results suggest that feeding the AAC diet to late-gestating beef cows stimulated programming effects on postnatal offspring growth and health compared with the CON diet. Therefore, supplementing late-gestating beef cows with an organic complexed source of Co, Cu, Zn, and Mn instead of no supplementation appears to optimize offspring productivity in beef production systems.

Keywords: beef cows, offspring, pregnancy, supplementation, trace minerals

INTRODUCTION

Nutritional management of beef cows during late-gestation, particularly energy and CP intake, impacts offspring performance via fetal programming (Funston et al., 2010; Bohnert et al., 2013). However, little is known about the effects of trace mineral status of late-gestating cows on offspring productivity. Trace minerals are essential for fetal development (Hostetler et al., 2003), and the fetus depends completely on the dam for proper supply of these elements (Hidiroglou and Knipfel, 1981). If maternal supply is inadequate, fetal development and postnatal performance might be impaired (Weiss et al., 1983). For examples Zn, Cu, Mn, and Co are required for adequate development of the fetal nervous, reproductive, and immune systems (Hostetler et al., 2003; Pepper and Black, 2011). Moreover, Cu concentration in bovine fetal liver is greater than maternal liver Cu concentration, suggesting that the maternal system shunts Cu to support fetal development (Gooneratne and Christensen, 1989). Therefore, we hypothesized that supplementing Cu, Mn, Zn, and Co to late-gestating cows will result in increased postnatal offspring productivity.

One strategy to enhance trace mineral status in cattle is to feed organic complexed sources (Spears, 1996). Hostetler et al. (2003) reported that Cu, Mn, and Zn concentrations in tissues of fetuses collected from sows supplemented with organic sources of these elements were greater compared with fetuses from sows supplemented with inorganic sources, which resulted in reduced fetal loss by 30 d of gestation. Hence, we also theorized that supplementing organic complexed sources of Cu, Mn, Zn, and Co to beef cows during late gestation is an alternative to further optimize postnatal offspring productivity. Based on these hypotheses, this experiment evaluated the effects of organic and inorganic Cu, Mn, Zn, and Co supplementation to beef cows during late gestation on performance and physiological responses of the offspring.

MATERIALS AND METHODS

Cow-calf management and dietary treatments. Eighty-four multiparous, non-lactating, pregnant Angus × Hereford cows (BW = 512 ± 6 kg, age = 5.1 ± 0.2 yr, BCS = 5.11 ± 0.04 according to Wagner et al., 1988) were assigned to the experiment at the end of their 2nd trimester of gestation (d 0). Cows were pregnant to AI using semen from a single Angus sire (n = 56) or pregnant to Hereford bulls via natural breeding (n = 28). On d -10, cows were ranked by pregnancy type (AI or natural service), BW, and BCS, and allocated to 21 drylot pens (4 cows/pen) in a manner that pens had

equivalent BW and BCS, and either 3 or 2 cows pregnant to AI. Pens were ranked by proportion of cows pregnant to AI or natural service, and alternatingly assigned to receive diets containing 1 of 3 treatments: 1) Cu, Co, Mn, and Zn sulfate sources (**INR**), 2) Cu, Mn, Co, and Zn complexed organic source (**AAC**; Availa®4; Zinpro Corporation, Eden Prairie, MN), or 3) no Cu, Co, Mn, and Zn dietary supplementation (**CON**). The INR and AAC sources were mixed with the corn, formulated to provide the same daily amount of Cu, Co, Mn, and Zn (based on 7 g/cow daily of Availa®4). Immediately after calving, cow-calf pairs were removed from their respective pens, and assigned to the general management of the research herd that included free-choice inorganic trace mineral supplementation.

Calf management

Preconditioning (d 283 to 328). Calves were weaned on d 283 of the experiment and transferred to a 6-ha meadow foxtail pasture for a 45-d preconditioning period as a single group. During preconditioning, calves received mixed alfalfa-grass hay (14% CP, 56% TDN; DM basis), water, and commercial mineral and vitamin mix for ad libitum consumption.

Growing (d 328 to 440) and finishing (d 440 until slaughter). On d 328, all calves were loaded into a commercial livestock trailer and transported to the growing lot (Top Cut; Echo, OR), where they remained for 112 d and managed as a single group. On d 440, calves were moved to an adjacent finishing lot (Beef Northwest; Boardman, OR), where they continued to be managed as a single group until slaughter at a commercial packing facility (Tyson Fresh Meats Inc., Pasco, WA).

Sampling

Two samples of all dietary ingredients fed to late-gestating cows (Table 1) were collected before the beginning of the experiment and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY).

Cows and newborn calves. Individual cow BW and BCS (Wagner et al., 1988) were recorded and averaged over 2 consecutive days prior to the beginning of the experiment (d -11 and -10), and 2 wk prior to the beginning of the estimated calving season (d 75 and 76). On d -10 and 75, liver biopsies were performed in all cows via needle biopsy. Within 3 h after calving and prior to the first nursing event, calf birth BW, birth date, and gender were recorded, whereas a liver sample was collected via needle biopsy. When feasible, the expelled placenta was retrieved and immediately rinsed with nanopure water for 5 min. The 5 largest cotyledons were dissected from each placenta, pooled and dried for 24 h at 65°C. Liver and cotyledon were subsequently stored at −80°C.

Preconditioning. Cow BW and BCS (Wagner et al., 1988) were recorded at weaning (d 283). Calf BW was recorded and blood samples were collected, via jugular venipuncture into commercial heparinized blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ), on d 283, 284, 286, 288, and 290 of the experiment. Calves were observed daily for bovine respiratory disease (**BRD**) symptoms, and treated when symptoms were observed.

Growing and finishing. Calf BW was recorded upon arrival at the growing lot (d 328) and the finishing lot (d 440). Calves were observed daily for BRD symptoms, and received medication according to the management criteria of the growing and finishing yards. At the commercial packing plant, carcass merits were collected upon slaughter.

Blood and tissue analysis. Liver and cotyledon samples were analyzed for concentrations of Co, Cu, Mn, and Zn by the Michigan State University - Diagnostic Center for Population and Animal Health. Blood samples were collected, centrifuged at 2,500 × *g* for 30 min for plasma collection and stored at -80°C on the same day of collection. Plasma samples were analyzed for haptoglobin and cortisol concentrations.

Table 1. Ingredient composition and nutrient profile of diets containing or not (**CON**) inorganic (**INR**) or organic (**AAC**) sources of supplemental Cu, Co, Mn, and Zn, as well as nutrient requirements (REQ; as % diet DM) of pregnant cows during last trimester of gestation.

Item	CON	INR	AAC	REQ[1]
Ingredients,kg/day (as-fed basis)				
Alfalfa hay	6.8	6.8	6.8	
Grass-seed straw	2.7	2.7	2.7	
Whole corn	2.3	2.3	2.3	
Macromineral mix	0.060	0.060	0.060	
Inorganic trace mix[2]	-	0.004	-	
Organic trace mix[3]	-	-	0.007	
DM intake, kg/d	10.8	10.8	10.8	11.0
Nutrient profile (DM basis)				
TDN, %	61	61	61	53
NEm, Mcal/kg	1.45	1.45	1.45	1.10
CP, %	14.4	14.4	14.4	7.8
Co, mg/kg	1.03	2.18	2.14	0.10
Cu, mg/kg	10.3	20.8	20.6	10.0
Mn, mg/kg	56	74	74	40
Zn, mg/kg	31	64	64	30

[1] Based on NRC (2000).
[2] Containing (DM basis) 500 g/kg of ground corn, 231 g/kg $ZnSO_4$, 147 g/kg $MnSO_4$, 114 g/kg $CuSO_4$, and 8 g/kg of $CoSO_4$.
[3] Availa®4 (Zinpro Corporation, Eden Prairie, MN).

Statistical analysis. All cow and calf variables were analyzed with pen as the experimental unit, and pen(treatment) and cow(pen) as random variables. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.), and Satterthwaite approximation to determine the denominator df for tests of fixed effects. Model statements for cow-related responses included the effects of treatment. Model statements for calf-related responses and placental cotyledons analysis included the effects of treatment, calf gender as independent covariate, as well as day and treatment × day interaction for plasma variables. In addition, DOF was included as an independent covariate for all finishing lot and carcass variables. The specified term used in the repeated statement for plasma variables was day, the subject was cow(pen), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the lowest Akaike information criterion. Results are reported as least square means, covariately-adjusted to calf gender and DOF when applicable, and separated using

PDIFF. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

RESULTS AND DISCUSSION

Nutrient composition and profile of diets offered to CON, INR, and AAC cows are described in Table 1. All diets provided adequate amounts of macro nutrients and trace minerals, based on the requirements of pregnant cows during last trimester of gestation (NRC, 2000). As expected, including the inorganic or organic sources of Cu, Co, Mn, and Zn equally increased concentration of these trace elements in INR and AAC diets (Table 1).

Cow parameters. Length of treatment administration, were similar ($P = 0.36$) among CON, INR, and AAC cows. Based on the experimental design, initial cow BW and BCS were also similar ($P \geq 0.41$) among treatments. No treatment differences were detected ($P \geq 0.61$) for BW change or pre-calving BW. Cows receiving CON gained less ($P \leq 0.05$) BCS during the last trimester of gestation compared with INR and AAC cohorts (main treatment effect, $P = 0.10$). However, such increase was insufficient to impact pre-calving BCS, which was similar ($P = 0.61$) among treatments and adequate to promote offspring productivity according to Bohnert et al. (2013). No differences were detected ($P \geq 0.38$) among CON, INR, and AAC cows for initial (d -10) liver Co, Cu, Mn, and Zn concentrations (Table 2), indicating that all treatment had similar and adequate Co, Cu, Mn, and Zn liver status prior to the beginning of the experiment. In pre-calving (d 75) samples, liver concentrations of Co, Cu, and Zn were greater ($P \leq 0.05$) for INR and AAC compared with CON, whereas INR cows had reduced ($P = 0.04$) liver Co, similar ($P = 0.62$) liver Zn, but greater ($P = 0.03$) liver Cu compared with AAC cows (Table 2). No treatment differences were detected ($P = 0.67$) on pre-calving liver Mn concentration. These results indicate that the INR and AAC diets successfully increased liver Co, Cu, and Zn concentrations, but not Mn.

No treatment effects were detected ($P \geq 0.40$) for cow BW and BCS at weaning, as well as BW and BCS change from pre-calving to weaning. No treatment effects were also detected ($P \geq 0.59$) for pregnancy rates to AI, bull breeding, and overall (AI + bull breeding).

Calf birth and weaning parameters. In the placental cotyledons (Table 3), Co concentrations were greater ($P \leq 0.05$) in AAC and INR compared with CON cows, and similar between INR and ACC cows ($P = 0.25$). Concentrations of Cu in placental cotyledons were greater ($P = 0.05$) in AAC compared with CON cows, and similar when comparing INR and CON cows ($P = 0.16$) or INR and ACC cows ($P = 0.51$). No treatment effects were detected for Mn and Zn concentrations in placental cotyledons ($P \geq 0.73$; Table 3). Upon calving, calves from INR and AAC cows had similar ($P = 0.21$) liver Co concentrations, but greater ($P < 0.01$) compared with calves from CON cows (Table 3). Liver Cu and Zn concentrations (Table 3) were greater ($P = 0.05$) in calves from AAC cows compared with cohorts from CON cows, but similar when comparing calves from INR and CON cows ($P = 0.19$) or calves from AAC and INR cows ($P = 0.30$). No treatment effect was detected for calf liver Mn concentration ($P = 0.43$). Given that the fetus relies completely on the dam for proper supply of trace minerals (Hidiroglou and Knipfel, 1981), treatment effects detected for cotyledon and calf liver Co concentrations suggest increased passage of this trace mineral through the placenta to the fetus when INR and AAC diets were offered to late-gestating cows instead of the CON diet (Pepper and Black, 2011). However, treatment differences in cotyledon Cu and calf liver Cu and Zn suggest that transfer of these elements from maternal to fetal tissues was only enhanced when the AAC diet was offered instead of the CON diet (Hostetler et al., 2003).

No treatment effects were detected ($P \geq 0.27$) for calving rate, calf birth BW (adjusted or not; BIF, 2010; Table 4), as well as kg of calf born per cow assigned to the experiment. At weaning, no treatment differences were detected ($P \geq 0.17$) for weaning rate and weaning age. Weaning BW and 205-d adjusted weaning BW (BIF, 2010) were greater ($P \leq 0.04$) for calves from AAC cows compared with calves from CON cows, and similar ($P \geq 0.18$) between calves from INR vs. AAC and INR vs. CON cows (Table 4). However, no treatment effects were detected ($P \geq 0.41$) for kg of calf weaned (actual or 205-d adjusted BW) per cow assigned to the experiment, which can be associated with the unexpected numerical decrease in weaning rate of INR cows. Weaning results indicate that supplementing late-gestating beef cows the AAC diet increased weaning BW by more than 20 kg compared with CON cows. These results suggest that feeding the AAC diet to late-gestating beef cows resulted in programming effects on postnatal offspring development. Conversely, CON cows gave birth and weaned a reduced ($P \leq 0.05$) proportion of male calves compared with INR and AAC cows (Table 4). Calf gender was not controlled in the experimental design because cows were assigned to treatments without knowledge of their fetal gender. For this reason, all calf variables were analyzed using calf gender as an independent covariate, whereas the treatment × gender interaction was not tested because the experimental units were not blocked by calf gender. Nevertheless, steers and heifers had similar ($P \geq 0.45$) weaning age (182 vs. 183 d, respectively; SEM = 3), weaning BW (223 vs. 224 kg, respectively; SEM = 5), and 205-d adjusted weaning BW (254 vs. 252 kg, respectively; SEM = 5) in the present experiment.

Calf preconditioning parameters. Upon weaning, a treatment × day interaction was detected ($P < 0.01$) for plasma cortisol. Cortisol concentrations increased in calves from all treatments after weaning (day effect, $P < 0.01$). However, cortisol concentrations were greater ($P < 0.01$) in calves from AAC and INR cows compared with CON cohorts, and similar between calves from AAC and INR cows ($P = 0.61$), 3 d after weaning (d 286). Accordingly, Long et al. (2010) reported that maternal nutrition during gestation influences adrenal steroidogeneses of the offspring. No treatment effects were detected for plasma haptoglobin concentrations, which increased (day effect; $P < 0.01$) for all treatments upon weaning (0.37, 1.31, 1.19, 0.93, and 0.72 μg/mL on d 283, 284, 286, 288, and 290, respectively; SEM = 0.05). These outcomes suggest that Co, Cu, Zn, and Mn supplementation to late-gestating cows impacted the steroidogenesis required to cope with the stress of weaning procedures in the offspring, without impacting the resultant acute-phase protein response (Carroll and Forsberg, 2007).

During the 45-d preconditioning, no treatment effects were detected ($P \geq 0.42$) for incidence of calves that required treatment for BRD, calf mortality, and ADG (Table 4), indicating that treatments did not influence calf preconditioning performance and health parameters despite treatment differences detected for weaning BW (Table 4) and plasma cortisol. At the end of preconditioning, BW was still greater ($P = 0.03$) for calves from AAC cows compared with calves from CON cows, and similar among calves from INR cows compared with AAC and CON cohorts ($P \geq 0.25$).

Calf feedlot and carcass parameters. During the growing lot phase, the proportion of calves treated for BRD symptoms was reduced ($P < 0.01$) in calves from AAC cows compared with calves from INR and CON cohorts (Table 5). During gestation, Zn, Cu, Mn, and Co are also essential for development of the fetal immune system (Hostetler et al., 2003; Pepper and Black, 2011), suggesting that feeding the AAC diet to late-gestating cows also resulted in programming effects on postnatal offspring health. Nevertheless, no treatment effects were detected ($P \geq 0.63$) for calf mortality and ADG in the growing lot (Table 5). Calf BW at the end of the growing lot phase was still greater ($P = 0.04$) for calves from AAC cows compared with calves from CON cows, and similar among calves from INR cows compared with AAC and CON cohorts ($P \geq 0.17$).

Similar to weaning outcomes, the proportion of AI-sired calves that were slaughtered did not differ ($P = 0.92$) among treatments, whereas a reduced ($P \leq 0.05$) proportion of male calves were slaughtered from CON cows compared with INR and AAC cohorts. However, calf gender was a significant covariate ($P \leq 0.04$) for all finishing and carcass variables. Therefore, all finishing and carcass results were adjusted to the significant ($P \leq 0.04$) calf gender covariate. No treatment effects were detected ($P \geq 0.59$) for calf ADG and BRD incidence (Table 5) during the finishing period, whereas no calf mortality was observed. Final finishing BW and HCW were again greater ($P = 0.05$; Table 5) for calves from AAC cows compared with calves from CON cows, and similar among calves from INR cows compared with AAC and CON cohorts ($P \geq 0.19$). No treatment effects were detected ($P \geq 0.46$) for any of the other carcass merit traits evaluated, or kg of carcass produced per cow assigned to the experiment. Collectively, these outcomes suggest that treatment effects on finishing BW and HCW were resultant from the greater weaning BW in calves from AAC cows compared with CON cohorts, while treatments and differences in finishing BW failed to impact carcass merit traits.

IMPLICATIONS

Supplementing beef cows during late gestation with organic or inorganic sources of Co, Cu, Zn, and Mn effectively increased cow liver concentrations of Co, Cu, and Zn compared with CON cohorts. Liver Cu and Zn concentrations in the neonatal calf were only increased in AAC compared with CON cows. Calves from AAC cows were > 20 kg heavier from weaning until slaughter and had reduced BRD incidence during the growing phase compared with calves from CON cows, which are suggestive of programming effects on postnatal offspring growth and health resultant from the AAC treatment (Funston et al., 2010). Nevertheless, results from this experiment are novel and suggest that supplementing late-gestating beef cows with an organic complexed source of Co, Cu, Zn, and Mn instead of no supplementation may be an alternative to optimize offspring productivity in beef production systems.

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Table 2. Liver concentrations of Co, Cu, Mn, and Zn of beef cows receiving diets containing or not (**CON**) inorganic (**INR**) or organic (**AAC**) sources of supplemental Cu, Co, Mn, and Zn during the last trimester of gestation.[1,2]

Item	CON	INR	AAC	*SEM*	*P* =
Co, ppm					
Initial (d -10)	0.29	0.28	0.27	0.01	0.38
Pre-calving (d 75)	0.21[a]	0.40[b]	0.44[c]	0.01	< 0.01
Cu, ppm					
Initial (d -10)	93	106	95	10	0.68
Pre-calving (d 75)	69[a]	155[b]	129[c]	9	< 0.01
Zn, ppm					
Initial (d -10)	171	176	171	5	0.70
Pre-calving (d 75)	211[a]	230[b]	235[b]	7	0.05

[1] INR and AAC cows received the same amount of Cu, Co, Mn, and Zn from sulfate sources or Availa®4 (Zinpro Corporation, Eden Prairie, MN). Within rows, means with different superscripts differ ($P \leq 0.05$).
[2] Liver samples were collected prior to the beginning of the experiment (initial; d -10), or 2 wk prior to the beginning of the calving season (pre-calving; d 75) via needle biopsy (Arthington and Corah, 1995). Concentrations of Co, Cu, Mn, and Zn were determined by the Michigan State University Diagnostic Center for Population & Animal Health.

Table 3. Concentrations of Co, Cu, Mn, and Zn in cotyledons and liver from newborn calves born from beef cows that received diets containing or not (**CON**) inorganic (**INR**) or organic (**AAC**) sources of supplemental Cu, Co, Mn, and Zn during the last trimester of gestation. [1,2]

Item	CON	INR	AAC	*SEM*	*P* =
Co, mg/kg					
Cotyledon	0.13[a]	0.20[b]	0.24[b]	0.03	0.02
Calf	0.09[a]	0.12[b]	0.13[b]	0.01	< 0.01
Cu, mg/kg					
Cotyledon	3.88[a]	4.75[ab]	5.12[b]	0.39	0.09
Calf	362[a]	428[ab]	450[b]	30	0.10
Zn, mg/kg					
Cotyledon	65	66	68	4	0.87
Calf	456[a]	562[ab]	660[b]	57	0.01

[1] INR and AAC cows received the same amount of Cu, Co, Mn, and Zn from sulfate sources or Availa®4 (Zinpro Corporation, Eden Prairie, MN). Within rows, means with different superscripts differ ($P \leq 0.05$).
[2] Cotyledon and calf liver samples (via needle biopsy; according to Arthington and Corah, 1995) were collected within 3 h after calving. Concentrations of Co, Cu, Mn, and Zn were determined by the Michigan State University Diagnostic Center for Population & Animal Health.

Table 4. Calving, weaning, and preconditioning outcomes from beef cows that received diets containing or not (**CON**) inorganic (**INR**) or organic (**AAC**) sources of supplemental Cu, Co, Mn, and Zn during the last trimester of gestation. [1]

Item	CON	INR	AAC	SEM	*P* =
Calving results					
% of male calves born	25.9[a]	58.3[b]	48.2[b]	9.5	0.05
Calf birth BW, kg	42.1	41.6	40.8	1.0	0.63
Adjusted calf birth BW,[2] kg	42.9	42.7	41.8	1.0	0.69
Weaning results					
% of male calves weaned	23.1[a]	58.3[b]	52.0[b]	9.6	0.04
Calf weaning BW, kg	212[a]	223[ab]	236[b]	6	0.04
Calf 205-d adjusted weaning BW,[2] kg	244[a]	252[ab]	263[b]	6	0.05
Preconditioning results					
Treated for BRD symptoms, %	34.9	36.4	31.5	11.7	0.95
End of preconditioning BW, kg	226[a]	236[ab]	246[b]	6	0.05
Preconditioning ADG, kg/d	0.23	0.14	0.19	0.04	0.34

[1] INR and AAC cows received the same amount of Cu, Co, Mn, and Zn from sulfate sources or Availa®4 (Zinpro Corporation, Eden Prairie, MN). Within rows, means with different superscripts differ ($P \leq 0.05$).
[2] Calculated according to BIF (2010).

Table 5. Feedlot performance and carcass characteristics of feeder cattle born from beef cows that received diets containing or not (**CON**) inorganic (**INR**) or organic (**AAC**) sources of supplemental Cu, Co, Mn, and Zn during the last trimester of gestation. [1]

Item	CON	INR	AAC	SEM	*P* =
Growing lot performance					
Treated for BRD symptoms, %	42.3[a]	59.1[a]	20.0[b]	9.6	0.02
BW at the end of growing lot, kg	352[a]	359[ab]	374[b]	8	0.09
Growing lot ADG, kg/d	1.11	1.09	1.13	0.04	0.86
Finishing lot performance					
Treated for BRD symptoms, %	0.0	5.2	4.4	3.6	0.37
BW at the end of finishing lot, kg	649[a]	663[ab]	680[b]	11	0.10
Finishing lot ADG, kg/d	1.89	1.95	1.97	0.05	0.57
% of male calves slaughtered	26.1[a]	59.1[b]	54.2[b]	10.2	0.05
Carcass characteristics					
HCW, kg	409[a]	418[ab]	428[b]	7	0.10
Backfat, cm	2.18	2.23	2.21	0.14	0.97
LM area, cm	96.0	95.8	98.4	1.8	0.53
Yield grade	3.89	4.06	3.94	0.19	0.81

[1] INR and AAC cows received the same amount of Cu, Co, Mn, and Zn from sulfate sources or Availa®4 (Zinpro Corporation, Eden Prairie, MN). Within rows, means with different superscripts differ ($P \leq 0.05$). Cattle were in the growing lot (Top Cut, OR) for 112 d, and moved to an adjacent finishing lot where they remained for an average of 153 d until slaughter at a commercial packing facility (Tyson Fresh Meats Inc., Pasco, WA).

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Immunological implications of pregnancy: A focus on inflammatory cytokines

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ABSTRACT: The present studies aim to determine expression of chemokine ligand twelve (CXCL12), CXCR4, and inflammatory cytokines in corpus luteum (CL) and fetal-maternal interface during early pregnancy and when CXCR4 signaling is inhibited in ewes. Human studies highlight CXCL12-CXCR4 signaling in regulating cytokine production and similar mechanisms may occur in livestock. Our laboratory reported activation of CXCL12-CXCR4 signaling at the ovine fetal-maternal interface but whether this axis is involved in modifying reproductive tissue or peripheral blood inflammatory responses is uncertain. We hypothesized CXCL12-CXCR4 signaling alters cytokine populations at the fetal-maternal interface and luteal microenvironment. To test this hypothesis, CL was collected from non-pregnant (NP, d 10 of estrous cycle) and pregnant ewes on d 20 and 25. In a separate study, we utilized AMD3100 to disrupt CXCR4 signaling to determine inhibition effects on fetal-maternal cytokines. Mini-osmotic pumps were surgically installed on d 12 of gestation and delivered AMD3100 or PBS into the uterine lumen ipsilateral to CL for 7 d. Endometrium, CL, and fetal membrane tissues were collected on d 23 of gestation. Gene expression of inflammatory cytokines was investigated using real time PCR. During gestation, pro-inflammatory cytokines increased ($P < 0.05$) in CL from pregnant compared to NP ewes. Similarly, CXCL12 and CXCR4 increased ($P < 0.05$) on d 20 of gestation in pregnant compared to NP ewes. In AMD3100-treated ewes, transcript for IL12 ($P < 0.01$) increased in caruncle, while tumor necrosis factor (TNF) ($P < 0.05$) increased in intercaruncular endometrium compared to control. Interleukin 10 ($P = 0.07$) and transforming growth factor beta 1 (TGFB1) ($P = 0.16$) transcript from treated ewe fetal membrane tended to increase compared to control. Immunofluorescence indicated IL10 localization to uterine luminal and glandular epithelium, TNF to uterine glandular epithelium and stroma, and IFNG to uterine stroma. Using flow cytometry, we established peripheral blood T lymphocytes are CXCR4-positive. Our results highlight the role CXCL12-CXCR4 signaling may play in regulating localized inflammation and immune cell trafficking in peripheral blood, contributing to pregnancy maintenance.
Key words: Chemokine ligand 12, chemokine receptor 4, corpus luteum, cytokines, inflammation

INTRODUCTION

Functional and structural integrity of reproductive tissues are dependent on balance of immune cell populations. Indeed, induced lymphopenia causes reduced plasma progesterone (P4) concentrations in cattle (Alila and Hansel, 1984), and immune cells including macrophages, T lymphocytes, dendritic cells, and natural killer cells are present in ovine endometrium (Segerson et al., 1991; Liu and Hansen, 1993; Mansouri-Attia et al., 2012), with macrophages accumulating throughout pregnancy (Tekin and Hansen, 2004). Recent evidence reveals immune cell and cytokine population changes in reproductive tissues are partially attributed to chemokines. Activation of chemokine ligand twelve (CXCL12)-CXCR4 signaling leads to synthesis of angiogenic factors (Molino et al., 2000), and aids in trophoblast cell survival, implantation, and angiogenesis of the placenta (Jaleel et al., 2004; Ren et al., 2012; Quinn et al., 2014). This ligand-receptor pair is quite crucial in driving immunological function through immune cell trafficking (Aluvihare et al., 2004; Wu et al., 2005). In vitro treatment of decidual immune cells with CXCL12 stimulates anti-inflammatory cytokine secretion and reduces pro-inflammatory cytokine production (Piao et al., 2012). Addition of anti-CXCR4 antibody abrogated these effects, suggesting disruption of the local anti-inflammatory environment when CXCR4 is antagonized (Piao et al., 2012). Whether similar mechanisms occur in livestock is uncertain. Our laboratory reported activation of CXCL12-CXCR4 signaling axis at the fetal-maternal interface in sheep, and this axis likely modifies reproductive tissue and/or peripheral blood inflammatory responses. We hypothesized CXCL12-CXCR4 signaling alters cytokine populations at the fetal-maternal interface, luteal microenvironment, and in peripheral blood mononuclear cells (PBMC). The present studies determined expression of CXCL12, CXCR4, and inflammatory cytokines in CL and the fetal-maternal interface during early pregnancy and when CXCR4 signaling is inhibited in ewes.

MATERIALS AND METHODS

Animals—days of early gestation

New Mexico State University Animal Care and Use Committee reviewed and approved all experimental procedures using animals. Estrus was synchronized in Western white face ewes as previously described (Quinn et al., 2014). Pregnancy was determined by serum P4 levels (> 1 ng/mL) with use of RIA as previously described (Coat-A-Count Siemens Medical Solutions Diagnostics, Los Angeles, CA; Schneider and Hallford, 1996). The interassay and intra-assay CVs were 7.8% and 6.0%, respectively. Ewes ($n = 4$ to 5/d) were anesthetized with sodium pentobarbital (20 mg/kg, intravenous) on d 20 or 25

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of gestation and on d 10 of the estrous cycle (NP, control ewes).

Animals—AMD3100 treatment

Fifteen Rambouillet-cross ewes received intravaginal controlled internal drug release (CIDR) inserts for 5 d to synchronize estrus and two injections of dinoprost tromethamine (5 mg intramuscular; Lutalyse; Pfizer, New York, NY) administered 4 h apart following CIDR removal. Ewes were mated by a fertile ram and randomly placed into experimental groups of either control (PBS, n = 7) or treatment (AMD3100, n = 8). On d 12 of gestation, ewes were anesthetized (5 mg xylazine and 100 mg ketamine, 1 mL intravenous) and maintained on isofluorane. Mini-osmotic pumps (2ML1, Alzet, Cupertino, CA, USA) were preloaded with AMD3100 (Selleckchem, US) or PBS (CON). The catheter attached to pump was introduced into the uterine lumen ipsilateral to CL, emptying treatment into the uterine lumen. Ewes were anesthetized with sodium pentobarbital (20 mg/kg intravenous) on d 23 of gestation.

Tissue and blood collection

Blood samples were collected on d 10 and 15 of pregnancy via jugular venipuncture into EDTA vacutainer tubes (Covidien, Dublin, Ireland). The reproductive tract was removed via mid-ventral laparotomy. Corpora lutea, endometrium (caruncle and intercaruncle; CAR and IC, respectively), and fetal membrane (FM) were collected with sterile technique, snap frozen in liquid nitrogen, and stored at -80°C for subsequent RNA isolation. Ewes were euthanized by exsanguination while under anesthesia.

RNA isolation and real timePCR (qPCR)

RNA was extracted from CL, CAR, IC, and FM, and real time PCR (qPCR) was completed as previously described (Quinn et al., 2014) using primers listed in Table 1. Data are represented by $2^{-\Delta Cq}$ values for days of gestation, and $2^{-\Delta\Delta Cq}$ values for study using AMD3100 treatment.

PBMC isolation

Equal amounts of whole blood were inverted with RPMI 1640 medium with 1% antimycotic/antibiotic (RPMI 1640), layered over Histopaque-1077 (10771, Sigma, St. Louis, MO, USA), and centrifuged at 500 x g for 45 min at room temperature with no brake. Peripheral blood mononuclear cell interface was isolated, diluted to 20 mL with RPMI 1640 medium, and centrifuged at 300 x g for 6 min at 4°C. All steps are at 4°C unless otherwise noted. Supernatant was removed and replaced with 1 ml RPMI 1640. Two mL red blood cell lysis buffer (0.15 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH = 7.2-7.4) was added and samples incubated on ice for 3 min until an additional centrifugation at 300 x g for 6 min. The remaining pellet of PBMC was resuspended in 750 µL flow buffer (Phosphate-buffered saline, 2% fetal bovine serum), counted using Countess Automated Cell Counter (Invitrogen, Carlsbad, CA, UA), and diluted to 10^6 cells/mL with flow buffer.

Table 1. Primer sequences for each gene of interest.

Gene	Reverse primer sequence	Forward primer sequence
GAPDH	5'-CGTTCTCTGCC TTGACTGTG-3'	5'-TGACCCCTTCA TTGACCTTC-3'
CXCL12	5'-GGTCAATGCAC ACTTGCCTA-3'	5'-CCTTGCCGAT TCTTTGAGAG-3'
CXCR4	5'-ATTTTCCTCCC GGAAGCAGG-3'	5'-GGGATCCGTATA TTCACTTCCGA-3'
IFNG	5'-TCTCCGGCCT CGAAAGAGAT-3'	5'-GGCTGATTCAA ATTCCGGTGG-3'
TNF	5'-TCAGGTAAAG CCCGTCAGTG-3'	5'-GTAGCCCACGT TGTAGCCAA-3'
IL12	5'-TCCAGAAGACA GACAATGCCC-3'	5'AGCCACGAAT GAGAGTTGCC-3'
IL10	5'-ACACCCCTC TCTTGGAGCAT-3'	5'-GGCGCTGTCA TCGTTTTCTG-3'
TFGB1	5'-CCGGAACTGA ACCCGTTGAT-3'	5'-AGAAGGCTTTC GCCTCAGTG-3'

Phenotype analysis

Aliquots of 100 µL of cell suspensions were placed in wells of a 96-well v-bottom plate, and stained for dual-color flow cytometry analysis using anti-CXCR4 PE conjugated antibody (12-9991, eBioscience, San Diego, CA, USA), and either anti-CD4 fluorescein isothiocyanate (FITC) conjugated antibody (MCA2213F, AbD Serotec, Raleigh, NC, USA) or anti-CD8 FITC conjugated antibody (MCA837F, AbD Serotec, Raleigh, NC, USA), and subsequently incubated for 45 min while protected from light. Stained cell suspensions were washed by centrifuging the plate at 300 x g for 7 min, removing supernatant and resuspending the pellet in additional 100 µL flow buffer, twice. Pellets were resuspended in 150 µL cold PBS, transferred to 1.7 mL microcentrifuge tubes, and 150 µL cold flow fixative (100% ethanol) was added dropwise while vortexing PBMC vigorously. Flow cytometry was performed within 24 h. Prior to flow cytometry analysis, cells were resuspended with an additional 200 µL PBS. Flow cytometry profiles were obtained on Accuri C6 (BS Biosciences, San Jose, CA, USA). Gates for identification of positive cells were established by analyzing other aliquots of cells incubated with control IgG conjugated with PE and FITC.

RESULTS AND DISCUSSION

Localized inflammation of CL in early pregnancy

As main contributor of P4 throughout early pregnancy, presence and maintenance of the CL is critical for conceptus implantation and pregnancy maintenance. The CL is characterized by a compact network of blood vessels that undergo angiogenesis, a process necessary to achieve steroidogenic capacity. Immune cell populations in the CL contribute to P4 synthesis and angiogenesis needed for CL

function (Miyamoto et al., 2013) through synthesis and secretion of cytokines.

In the current study, pro-inflammatory cytokine expression increased on d 20 and 25 for IL12, d 25 for interferon gamma (IFNG), and d 20 for tumor necrosis factor (TNF) ($P < 0.05$, Fig. 1a and b) compared to NP ewes. Conversely, anti-inflammatory cytokines transforming growth factor beta 1 (TGFB1) and IL10 were expressed in CL from pregnant and NP ewes, but did not differ ($P > 0.05$, data not shown). Skarzynski et al. (2007) demonstrated infusion of high concentrations of TNF in cattle prevents luteolysis and prolongs CL function. Additionally, T cytotoxic (Tc) lymphocytes constitute 45% of lymphocytes present in bovine CL at d 10-12 of the estrous cycle (Poole and Pate, 2012), and macrophages are also present during CL development in cattle (Penny et al., 1999); both of these cell types synthesize and secrete pro-inflammatory cytokines.

Activation of the CXCL12-CXCR4 signaling axis is key in regulating immune cell trafficking to the fetal-maternal interface, and similar immune cell migration may occur in CL. Chemokine ligand twelve is secreted by luteinizing granulosa cells and increases in CXCL12 correlate with increased P4 production (Kryczek et al., 2005; Nishigaki et al., 2013). Gene expression for CXCL12 ($P < 0.05$, Fig. 2a) and CXCR4 ($P < 0.05$, Fig. 2b) temporally increased on d 20 of gestation compared to NP and d 25 ewes. The concurrent increases in gene expression for CXCL12 and CXCR4 suggests this signaling axis may enhance CL growth and angiogenesis with assistance from P4, underscoring the importance of CXCL12-CXCR4 signaling in successful pregnancy maintenance.

The simultaneous increases in pro-inflammatory cytokines, CXCL12, and CXCR4 in early pregnancy suggest T cell regulation important for CL maintenance, however the overall role of pro-inflammatory cytokines in CL must be further characterized. Future research aims to elucidate CXCL12 and CXCR4 involvement in luteal function using in vitro methods.

CXCR4 inhibition in reproductive tissues

In humans, the conceptus attaches and protrudes into maternal endometrium, which responds to this insult with a powerful inflammatory response. Immune cell populations are present and dynamic, aiding in spiral artery remodeling, uterine epithelium repair, cellular debris removal, and regulation of placental lactogen concentrations in humans (Abrahams et al., 2004; Kzhyshkowska et al., 2008; Mor and Koga, 2008; Robson et al., 2012). Ruminants in contrast to humans and rodents exhibit a lesser invasive cotyledonary placenta characterized by multiple attachments of fetal chorion (cotyledons) to aglandular caruncles of maternal endometrium to create placentomes. Though deemed less invasive, placentome development in ruminants is decidedly similar to decidualization in rodents and humans, depending on local extracellular and cell membrane modifications that facilitate trophoblast attachment (Johnson et al., 2001; von Wolff et al., 2004; Carson et al., 2006). Much of our knowledge about peri-implantation immune responses of livestock has been in cattle (reviewed by Bauersachs and Wolf, 2015), and still leaves more to be understood regarding local inflammation.

The chemokine-receptor pair CXCL12 and CXCR4 maintains a biological relationship integral to pregnancy maintenance. In this study, we investigated cytokine populations and CXCL12-CXCR4 signaling at the fetal-maternal interface in vivo by inhibiting CXCR4 signaling in ovine uterus using the potent CXCR4 antagonist AMD3100. Pro-inflammatory cytokines IL12 ($P < 0.05$) and TNF ($P = 0.07$) increased in CAR tissue of uterine horn contralateral to pump installation (CAR) and CAR tissue ipsilateral to pump installation (CAR-P) of AMD3100-treated ewes compared to CON, respectively, while all other inflammatory cytokines did not differ (Fig. 3a and b). Intercaruncular tissue yielded similar results with elevated TNF following AMD3100 treatment ($P < 0.05$, Fig. 4). All transcripts were detected in IC-P, but did not differ ($P > 0.05$, data not shown). AMD3100 antagonizes CXCR4 through high-affinity binding and impedes binding and function of CXCL12 (Rosenkilde et al., 2004). Thus, the demonstrated increase in pro-inflammatory cytokine expression in CAR and IC may be due to an inability of the CXCL12-CXCR4 signaling axis to modulate immune cell populations, inherently affecting endometrial cytokine balance.

In contrast to CAR and IC, expression of anti-inflammatory cytokines IL10 ($P = 0.07$) and TGFB1 ($P = 0.16$) tended to increase in FM of AMD3100-treated ewes compared to CON, and TNF and IFNG were not expressed in CON or treated ewes (Fig. 5). However, in addition to antagonizing CXCR4, AMD3100 also acts as an allosteric agonist to CXCR7, increasing CXCL12 binding to CXCR7 (Kalatskaya et al., 2009), and the CXCR7-TGFB1 axis mediates migration, invasion, and endothelial-mesenchymal transition (Wu et al., 2015). Therefore, CXCL12 may be acting through CXCR7 to initiate this increase in anti-inflammatory cytokines.

Continuing with the trend found in CAR and IC, TNF increased ($P < 0.05$) in CL of AMD3100-treated ewes on d 23 of gestation, but other inflammatory cytokines did not differ (Fig. 6). AMD3100 may be traveling systemically to reach the CL and elicit similar effects as in maternal endometrium. Alternatively, immune cell populations at the fetal-maternal interface may be altered by blockage of CXCL12-CXCR4 signaling in utero, and travel to CL with altered cytokine production. Additionally, transcript for CXCL12 decreased in CL of treated ewes compared to CON ($P < 0.05$, Fig. 7). This result provides further complexity regarding signaling mechanisms affected by AMD3100 treatment at the fetal-maternal interface.

CXCR4 inhibition in peripheral blood

A closely regulated population of immune cells is present in circulating blood of pregnant women and is implicated in successful acceptance of the semiallogeneic conceptus. Functional T regulatory cells are upregulated in early human pregnancy (Somerset et al., 2004), while Tc cells do not differ in number, but reduce pro-inflammatory functionality by decreasing synthesis of TNF compared to non-pregnant women (Meggyes et al., 2014). This

phenomenon is not well understood in humans and to lesser extent in sheep, but ovine pregnancy mirrors human pregnancy in placental development, metabolic functions, and nutrient transport (reviewed by Barry and Anthony, 2008). In the current study, antibodies to lymphocyte surface proteins cluster of differentiation (CD) 4 and CD8 as well as the receptor CXCR4 were used to determine expression of CXCR4 on T cell subsets in peripheral blood.

Abundance of $CD8^+CXCR4^+$ T lymphocytes decreased in peripheral blood of AMD3100-treated compared to CON ewes on day 15 of pregnancy ($P < 0.05$, Fig. 9). Abundance of $CD4^+CXCR4^+$ cells remained stable in AMD3100 and CON ($P > 0.05$, data not shown). This is surprising, as CXCL12 signaling in monocytes stimulates activation to an anti-inflammatory M2 macrophage phenotype (Sanchez-Martin et al., 2011), but inhibition of CXCL12-CXCR4 signaling by AMD3100 doesn't appear to encourage differentiation of cells with a pro-inflammatory phenotype.

We initially hypothesized the CXCL12-CXCR4 signaling axis alters immune cell populations in peripheral blood. Our results provide compelling evidence in favor of this hypothesis, as $CD8^+CXCR4^+$ populations were reduced in AMD3100-treated ewes. Chemokine receptor 4 signaling may therefore be involved in regulating circulating immune cell populations during early pregnancy; however, future studies must further elucidate modulation of specific cell types as well as the mechanism of action.

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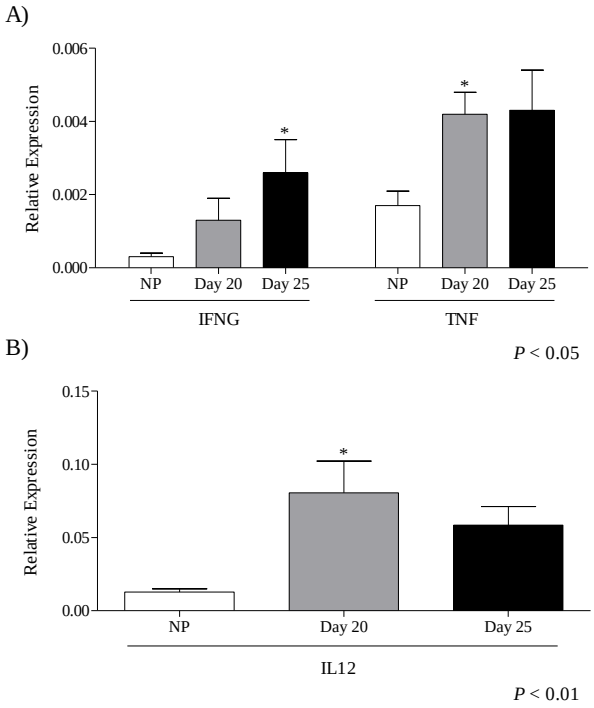

Figure 1. Expression of mRNA for interferon gamma (IFNG) and tumor necrosis factor (TNF) increased on either d 20 or d 25 of gestation compared to non-pregnant (NP) ewes ($P < 0.05$, A). Interleukin 12 expression increased on d 20 and 25 of pregnancy compared to NP ewes ($P < 0.05$, B). Data are represented by $2^{-\Delta Cq}$. Data represents the mean ± SEM with significant differences between NP denoted by asterisk.

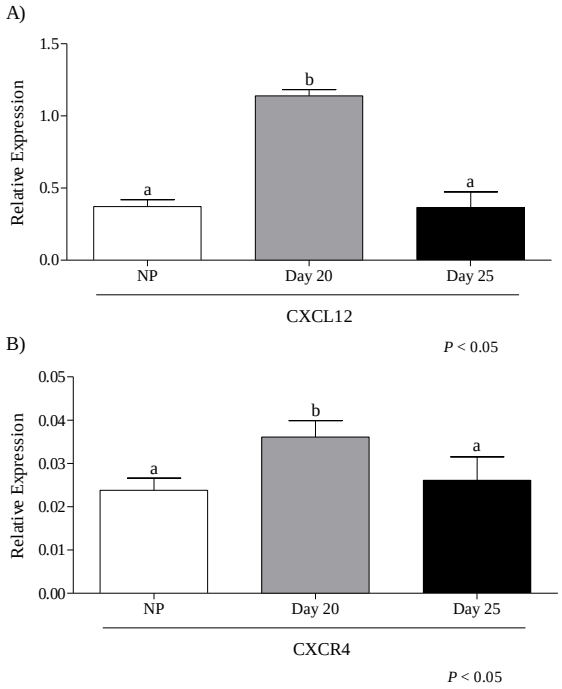

Figure 2. Expression of mRNA for chemokine ligand 12 (CXCL12) and chemokine receptor 4 (CXCR4) increased on d 20 compared to non-pregnant (NP) and d 25 ewes ($P < 0.05$). Data are represented by $2^{-\Delta Cq}$. Data represents the mean ± SEM with significant differences denoted by different letters.

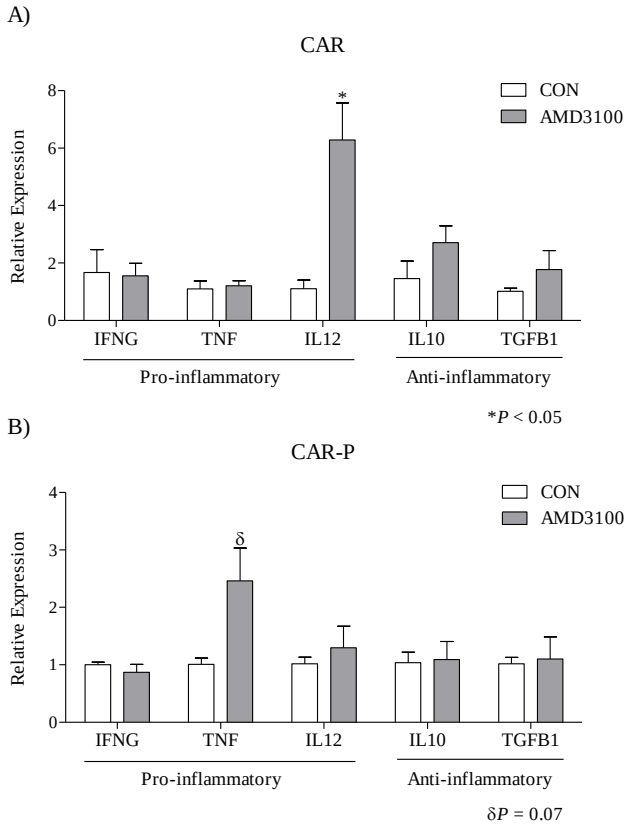

Figure 3. Expression of mRNA for IL12 increased in caruncle uterine horn contralateral to pump installation (CAR) of ewes treated with AMD3100 compared to control (CON) ($P < 0.05$, A). Tumor necrosis factor (TNF) tended to increase in CAR of uterine horn ipsilateral to pump installation (CAR-P) of AMD3100 compared to CON ($P = 0.07$, B). Mini-osmotic pumps were installed to release treatment into the uterine horn ipsilateral to the corpus luteum (CL) on d 12 of pregnancy. Data are represented by $2^{-\Delta\Delta Cq}$. Data represents the mean ± SEM with significant difference denoted by asterisk, and tendency to differ denoted by delta.

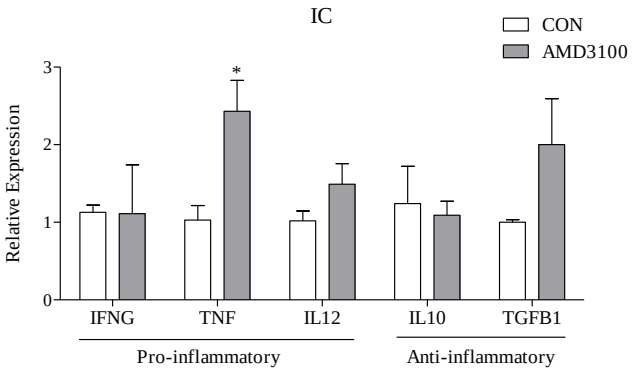

Figure 4. Expression of mRNA for tumor necrosis factor (TNF) increased in intercaruncle of uterine horn contralateral to pump installation (IC) of ewes treated with AMD3100 compared to control (CON) ($P < 0.05$). Data are represented by $2^{-\Delta\Delta Cq}$. Data represents the mean ± SEM with significant difference denoted by asterisk.

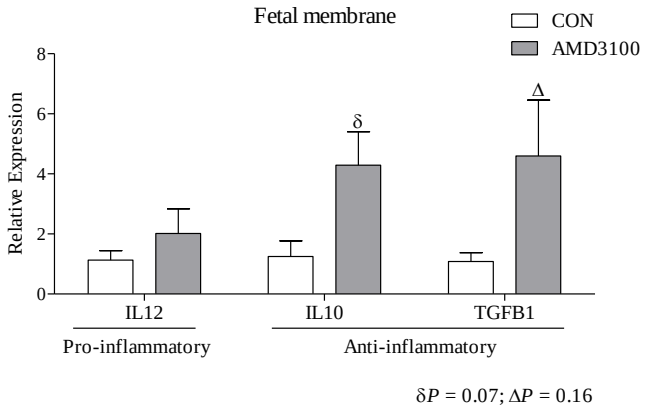

Figure 5. Expression of mRNA for IL10 ($P = 0.07$) and transforming growth factor beta 1 (TGFB1) ($P = 0.16$) tended to increase in fetal membrane of ewes treated with AMD3100 compared to control (CON). Data are represented by $2^{-\Delta\Delta Cq}$. Data represents the mean ± SEM with tendency to differ denoted by delta.

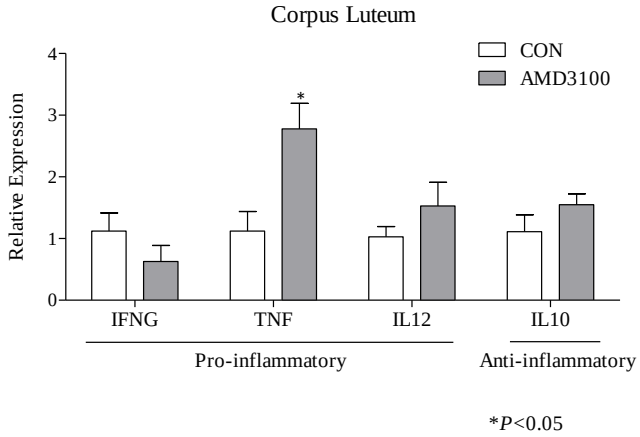

Figure 6. Expression of mRNA for TNF increased in corpus luteum (CL) of ewes treated with AMD3100 compared to control (CON). Data are represented by $2^{-\Delta\Delta Cq}$. Data represents the mean ± SEM with significant difference denoted by asterisk.

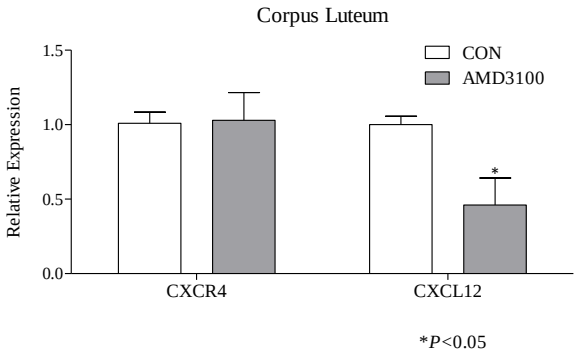

Figure 7. Expression of mRNA for chemokine ligand 12 (CXCL12) decreased in corpus luteum (CL) of ewes treated with AMD3100 compared to control (CON). Data are represented by $2^{-\Delta\Delta Cq}$. Data represents the mean ± SEM with significant difference denoted by asterisk.

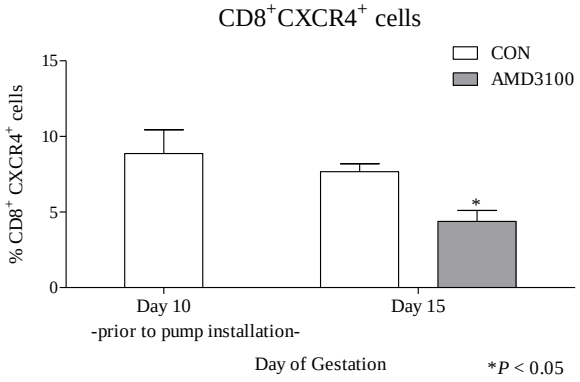

Figure 8. Percentage of circulating $CD8^+CXCR4^+$ T lymphocytes reduced ($P < 0.05$) on d 15 of gestation in AMD3100-treated ewes ($n = 4$) compared to d 15 control ewes (CON, $n = 4$). D 10 indicates cell populations prior to pump installation, as mini-osmotic pumps were installed on d 12 of pregnancy. Data represents the mean $\pm$ SEM with significant difference denoted by asterisk.

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