PROCEEDINGS Volume 65

WESTERN SECTION AMERICAN SOCIETY OF ANIMAL SCIENCE



San Angelo, Texas July 25–27, 2014

PROCEEDINGS Volume 65



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2013-2014 WSASAS COMMITTEES

EXECUTIVE

- J. Taylor, President USDA, ARS, Dubois, ID
- J. Berardinelli, President-Elect Montana State University
- G. Duff, Past President Montana State University
- M. Salisbury, Secretary-Treasurer Angelo State University, TX
- C. Larson, Industry Director Zinpro Corporation, Eden Prairie, MN
- J. Whittier, ASAS Director Colorado State University
- S. Ivey, A&C Committee Chair, New Mexico State University
- K. Quinn, Graduate Student Representative New Mexico State University
- J. Graves, Graduate Student Representative New Mexico State University

AWARDS (3 YEAR TERM)

- *, ‡G. Duff Montana State University (14)
- R. Endecott Montana State University (14)
- S. Ivey New Mexico State University (14)
- M. Benson Washington State University (15)
- T. Engle Colorado State University (15)
- S. Lake University of Wyoming (16)
- K. Vonnahme North Dakota State University (16)

BEEF SYMPOSIUM (3 YEAR TERM)

- *S. Lake University of Wyoming (14)
- J. Hall University of Idaho (14)
- D. Zobell Utah State University (15)
- R. Cooke Oregon State University (15)
- D. Faulkner University of Arizona (15)
- A. Grove AG Research LLC, White Sulphur Springs, MT (16)
- B. Neville North Dakota State University (16)

ADVISING AND COORDINATING (3 YEAR TERM)

- *S. Ivey New Mexico State University (14)
- [‡]J. Graves New Mexico State University (14)
- ‡J. Berardinelli Montana State University (14)
- R. Waterman USDA, ARS, Miles City, MT (14)
- A. Ahmadzadeh University Idaho (14)
- T. Engle Colorado State University (15)
- J. Caton North Dakota State University (15)
- H. Neibergs Washington State University (16)
- K. Cammack University of Wyoming (16)

PAPER COMPETITION (2 YEAR TERM)

- *R. Ashley New Mexico State University (14)
- M. Mousel USDA, ARS, Pullman, WA (14)
- C. Schauer North Dakota State University (14)
- J. Ahola Colorado State University (14)
- K. Johnson Washington State University (15)
- B. Alexander University of Wyoming (15)
- J. Thomson Montana State University (15)
- B. Glaze University of Idaho (15)

ACADEMIC QUADRATHLON

- *D. Rule University of Wyoming
- J. Lamb University of Idaho
- S. Sotto-Navaro New Mexico State University
- B. Bowman Utah State University
- H. Han Colorado State University
- S. Archibeque Colorado State University
- R. Endecott Montana State University
- M. Kennedy Oregon State University

NECROLOGY

‡G. Duff – Montana State University (14)

NOMINATING

- ‡, *G. Duff Montana State University (16)
- ‡A. Roberts USDA, ARS, Miles City, MT (15)
- ‡D. Crews Colorado State University (14)

ASAS WESTERN SECTION YOUNG SCHOLARS PROGRAM

- *, ‡C. Larson Zinpro Corporation, Eden Prairie, MN (16)
- M. MacNeil USDA, ARS (retired), Miles City, MT (14)
- G. Lardy North Dakota State University (14)
- J. Whittier Colorado State University (15)
- G. Moss University of Wyoming (15)
- R. Ashley New Mexico State University (16)
- P. Hatfield Montana State University (16)

^{* =} Chair

[‡] = Mandatory, not appointed

^{§ =} Not appointed by WSASAS President

Minutes of the WSASAS Annual Business Meeting

June 21, 2013 • Montana State University Prepared by James G. Berardinelli, Secretary-Treasurer

The Annual Business Meeting of the General Membership, Executive Committee and the ASAS Executive Staff was held at Montana State University, Bozeman, MT in room 138 Animal Bioscience Building to report, discuss and make decisions and recommendations of the agenda items listed below and other business of the Western Society of the American Society of Animal Science.

Call to Order

The meeting was called to order by Glenn Duff, President, at 07:30 am (MDT).

Approval of the Agenda

The agenda (Appendix 1) was approved by general consent.

Acceptance of 2012 WSASAS Business Meeting Minutes After a call for additions or amendments to the Minutes of the 2012 WSASAS Business Meeting a motion was made by Bret Taylor and seconded by Jack Whittier to accept the Minutes as published in the 2013 Western Section of the ASAS Proceedings, Volume 64, page xi. Glenn Duff asked for comments/questions; none were made. Motion passed unanimously.

2013 Financial Report

Glenn Duff presented an overview of the Financial Report. Stated that there was estimated income of approximately \$38,000 most of which from the registration. He indicated that expenses for Annual Meeting were expected to be lower than expected since meeting building, room, and equipment cost were basically "free", except for rental of space (rooms) for the Sheep symposium and Graduate Student Paper Competition presentations at the Museum of the Rockies. He stated that "…we should come out ahead on this year's meeting" financially. Lastly, he indicated that once the Executive Committee received that Financial Report from ASAS that it would be sent to the members. A copy of the report is presented in of these minutes (Appendix 2).

2013 WSASAS Meeting Report

Jim Berardinelli, Secretary-Treasurer, reported that there were a total of 139 registered attendees for the Annual Meeting: 80 Professional; 7 Retirees; 39 Graduate Students and 2 Undergraduate Students. Both the Beef and Sheep Symposia we well attended with registered attendees of 67 and 28, respectfully, and many other local and regional clientele (>120 individuals for the Sheep Symposium). There were 100 presentations at the Meeting: 72 and 28 oral and poster presentation, respectively. We had 21 Graduate Student Paper Competition Paper presentations. Lastly, he reported that there was a total of 79 Proceedings papers.

Glenn Duff reported that there were more than 125 attendees for the Awards Banquet.

Necrology Committee Report

Andy Roberts, Chair, Past-President, USDA, ARS, Ft. Keogh LARRL, Miles City, MT (Presented by Dennis Hallford, New Mexico)

Members of the WSASAS or those that were closely associated with the WSASAS mission that passed away during 2012-2013. They were:

1. John Smith, Dairy Extension Specialist, University of Arizona, Tucson

- 2. David Graham, County Extension Agent, New Mexico
- 3. Dean Fretchner

The report was followed by a moment of silence in memory of our deceased members and friends of the WSASAS.

Nominating Committee Report

Andy Roberts, Chair, Past-President, reported the nominees to be placed on the 2013 election ballot for officers. Nominees and elected officers for 2014 were:

- J. Bret Taylor President
- Jim Berardinelli President Elect
- Mike Salisbury Secretary
- James Grave –Graduate Student Representative. There were a total of 4 graduate students nominated for this position.
- Jack Whittier ASAS National Director. Holly Neibergs was the other nominee

Andy asked if there were any questions or comments regarding this report; there were none made.

Beef Symposium Committee Report

John Hall, Chair, University of Idaho (Presented by Glenn Duff, Montana State University) prepared the report presented in these minutes (Appendix 3). Glenn gave an overview of the purpose of and the details of operations of ranches in Southwest Montana that were visited in this year's Beef Symposium "Bus tour". Then he listed the speakers that gave oral presentations related to the topics associated with management, operation, and finance of these ranches.

Glenn also reported that the Sheep Symposium was very well-received by producers in Montana. Glenn asked that were thank and appreciate the corporative spirit of the members of the WERA-39 Committee.

Academic Quadrathlon Committee Report

Dan Rule, Chair, University of Wyoming, presented the report. There were 9 teams representing: USU, CSU, OSU, UA, UWY, NMSU, BYU-I, MSU, and Cal-Chico. This was the highest number of teams in over 20 years. The winner of this year's AQ competition was Montana State University. They will be listed 1st on the AQ winners "Travel Trophy". The trophy will stay at MSU for the next year and then taken to Angelo State University for next year's AQ competition; then travel to the home university of the winning team.

Dan commented on the importance of undergraduate students on these teams that had the opportunity to attend the scientific sessions. Students commented that from these experiences that see how what they learn comes from scientific research from students much like themselves. This activity underscores the importance of the AQ competition to the WSASAS.

Also, Dan stated that he will work with Mike Salisbury to prepare for the 2014 AQ Competition at Angelo State University, not only in regard to facilities and services, but in terms of travel and accommodations for members of participating teams.

A special thank you was given to Rachel Endecott for her outstanding activities and service for hosting the 2013 AQ event at MSU.

Lastly, Glenn Duff, Andy Roberts, Jack Whittier, and the rest of the assemblage thanked Dan Rule for his long-standing and outstanding efforts and service to the WSASAS AQ activities and for developing the concept of the "Traveling trophy".

Awards Committee Report

Bret Taylor, Chair, President-Elect, listed the WSASAS award winners for 2013, then presented the report (Appendix 4). He encourage the members to nominate members of the WSASAS for the awards. Bret asked if there were any questions or comments. Jim Berardinelli commented that people who win awards will probably asked to serve on the Awards Committee during the next cycle. Glenn called for other comments; none were made.

Applied Paper Awards

Connie Larson, Chair, Zinpro Corporation, stated that this was the 13th year for this award and there were 13 papers submitted to the Committee for consideration. She then presented the report (Appendix 5). There were no comments or questions regarding her report.

Young Scholars Recognition

Connie Larson, Chair, Zinpro Corporation, present the report for the 1st annual award for the Young Scholars Recognition Program (Appendix 6). She made the comment that we should encourage Ph.D. students to apply. The Committee suggested that in the following years we consider giving 1 Ph.D. and 2 M.S. students these awards. Furthermore, the web site information, access to and deadlines for nominations should be improved so that the process could be made clearer. She acknowledge the high level of service and patience of the Committee. Glenn Duff encouraged the members to nominate these individuals for the awards.

Graduate Student Competition Committee Report

Chad Mueller, Chair, Oregon State University, presented the report (Appendix 7). There was discussion as to whether or not the Institutional Graduate Student Award should be awarded this year because of a funding issue. Chris Schauer put forth the idea that perhaps we should re-institute this award and give a monetary or some type of trophy (similar to the Traveling Trophy for the AQ). Dennis Hallford moved that we institute a "Traveling Trophy" in the forthcoming years for the Institutional Graduate Student Award of the WSASAS. Bret Taylor seconded this motion. There was an amendment to the motion to give this award this year (2013). Connie Larson thought that Zinpro Corp. would sponsor such a trophy and Bret asked that we acknowledge Zinpro Corp. for their continued support to WSASAS activities. The motion was passed unanimously. Jack Whittier suggested that Dan Rule assist in developing the trophy.

Andy Roberts reminded the members that the Executive Committee made a policy that any graduate student that submitted

Advisory and Coordinating Committee Report

Mike Salisbury, Chair, San Angelo State University, stated that there was no new business or consideration for the Committee this past year due to re-structuring of the Executive Committee and other Committees of WSASAS, and the duties and responsibilities of the members of these Committees. Thus, no written report is given in these minutes. Western Section Proceedings Publication Committee Dennis Hallford, Chair, New Mexico State University, presented the report (Appendix 8). Dennis Hallford asked for questions and proceedings. There was a question as to whether if a revision of the reviewed abstract was necessary, would the authors then have the opportunity to revise their Proceedings paper in this new "platform". Mark Peterson made a motion we do not reject the graduate student paper competition abstract and allow them to go through the entire process. Mike Salisbury seconded the motion. Glenn thought that they could be rejected if they are of insufficient quality based on our standards. There was discussion as to how we should handle reviews and revisions. The motion was tabled and Glenn Duff recommended that the matter be sent to the Advising and Coordinating Committee for evaluation and recommendation. There was a general consensus to accept Glenn's recommendation.

Report from the ASAS President.

James L. Sartin, ASAS President, Auburn University, reported on the "State of the American Society of Animal Science" and changes in the Society over the past year. He summarized the changes in the technology for publications and databases of the Society. He stated that there were 5,802 members in the Society and that 26.8% of these were not from the U.S.; he expects this percentage to increase in the forthcoming years. He outlined new membership for the Society and the "Junior Animal Scientist" program and encourage everyone to support this program. He stated that the Journal has been "modernized" and new programs related to "Animal Frontiers" and ASAS "Taking Stock" in Washington, D.C. Web site is doing extremely well in term of number of "hits". Explained how our social media exposure is increasing and doing well. Lastly, he explain how the Society is attempting to influence policy in Washing, D.C. and the impact of "pods"; local specialists that can respond to changes in a timely manner in that particular area. There will be a new journal, "Animal Science and Natural Resource Communication", with emphasis on education. He did introduce the "coffee" initiative; asking that we buy into this new initiative. Then he reviewed the early registration for annual national meeting program and the Foundation program and their growth and awards. Lastly, he gave an overview of the financial state of the Society. The questions was asked if we were officially "divorced from FASS. He said we were still invested in the association but we will change the services from them to ASAS new contractor to decrease cost (~50% less) and time to publication to less than 6 months. Jacelyn indicated that platform contracts are for 1 year and then a decision is made to stay or look elsewhere for platforms that we work for the Society.

New Business: Assignment of Roles of Executive Committee

Bret Taylor, President Elect, USDA, ARS, Dubois, ID, reported on the May 8th, Executive Board meeting related to changes in the duties and responsibilities of the Officers of WSASAS. These can all be found on the WSASAS web site. Glenn asked for questions of comments; there were none.

New Abstract/Proceedings Deadlines

Glenn Duff, President, Montana State University, reported that the new system for submission of abstracts and Proceedings papers is now in place. Jacelyn indicated what the timeline of these would be. Jim Berardinelli commented that this year we will be probably be a "learning" year and we will be flexible for this timeline; it will be more streamlined in the next year. Glenn asked for questions or comments. Dennis Hallford asked if this change to the new system required a motion and vote. Glenn stated that it did not, but required only the approval of the Executive Committee, that had already approved the change in May.

Strategic Planning

Glenn Duff, Montana State University, reported that the Industry Representative is the chair of the Strategic Planning Committee of WSASAS, Connie Larson. One issue is the "boundaries of WSASAS. Traditionally, western states, but now we have members in Texas (Southern) and North Dakota. Glenn asked for volunteers to serve on the Committee and to contact her if you are willing to do so. Jacelyn had copies of the ASAS Strategic Plan to use as a starting place. These documents are on the ASAS web site.

Transfer of the Gavel

Glenn Duff transferred the gavel to President Elect, J. Bret Taylor. Bret Taylor, President, then presented Glenn Duff, Past President, with a WSASAS plaque in recognition of his excellent service as an officer in the WSASAS.

Adjournment

Before adjourning, Kelsey Quinn asked that we thank Eli Camacho for her service as Graduate Student Representative to WSASAS.

Rachel Endecott moved for adjournment. The motion was seconded by Jim Berardinelli. The motion passed unanimously.

Bret Taylor adjourned the meeting at 8:47 am.

BUSINESS MEETING AGENDA

Western Section, American Society of Animal Science Friday, June 21, 2013 138 ABB; Montana State University

Call to Order Approval of the Agenda Acceptance of 2012 WSASAS Business Meeting Minutes 2013 Financial Report 2013 WSASAS Meeting Report Necrology Committee Report - Andy Roberts, Past-President, USDA, ARS, Ft. Keogh LARRL, Miles City, MT Nominating Committee Report - Andy Roberts, Past-President, USDA, ARS, Ft. Keogh LARRL, Miles City, MT Beef Symposium Committee Report - John Hall, University of Idaho Academic Quadrathlon Committee Report - Dan Rule, University of Wyoming Awards Committee Report - Bret Taylor, President-Elect, USDA, ARS, Dubois, ID Applied Paper Awards – Connie Larson, Chair, Zinpro Corporation, Eden Prairie, MN Young Scholars Recognition - Connie Larson, Zinpro Corporation, Eden Prairie, MN Graduate Student Competition Committee Report - Chad Mueller, Oregon State University Advisory and Coordinating Committee Report – Mike Salisbury, San Angelo State University Western Section Proceedings Publication Committee – Dennis Hallford, New Mexico State University Report from the ASAS President - James L. Sartin, Auburn University **New Business:**

Assignment of Roles of Executive Committee – Bret Taylor, USDA Dubois, ID New Abstract/Proceedings Deadlines – Glenn Duff, Montana State University Strategic Planning – Glenn Duff, Montana State University

Transfer of the Gavel

AMERICAN SOCIETY OF ANIMAL SCIENCES STATEMENT OF ACTIVITIES WESTERN SECTION

		YTD		YTD		YTD		YTD
	1:	2/31/13	1	2/31/12	1	2/31/11	12	2/31/10
Revenue and Support	_							
Ticketed Events	\$	9,875	\$	3,360	\$	4,139	\$	2,610
Donations-Awards		5,625		1,425		1,250		4,150
Proceedings		8,385		10,745		9,090		11,708
Registrations		20,146		1,566		20,551		1,595
Investment Earnings Gain (Loss)		6,922		5,246		(873)		5,066
Total Revenue and Support		50,953		22,342		34,157		25,129
Expenses								
Programs/ Registration		1,297		2,292		221		-
Awards/Plaques		13,898		6,235		5,950		6,000
Convention Center		17,125		1,716		10,371		3,048
Marketing		3,302		865		3,273		-
Proceedings		4,021		2,348		1,407		448
Postage, Shipping & Supplies		363		17		984		1,705
Miscellaneous		7,183		2,707		7,895		4,013
Insurance		-		195		111		225
Staff Support		6,553		3,784		10,802		6,406
Total Expenses		53,742		20,159		41,014		21,845
Change in Net Assets		(2,789)		2,183		(6,857)		3,284
Net Assets, Beginning of Period	_	53,732		51,549		58,405		55,120
Net Assets, End of Period	\$	50,943	\$	53,732	\$	51,548	\$	58,404

2013 WSAS Beef Symposium

Pre-conference Report to WSAS Executive Committee Prepared by John B. Hall

The 2013 WSAS Beef Symposium Committee met several times by conference call or e-mail in early 2013 to discuss the content of the beef symposium. It was decided that this year's Beef Symposium combine a tour of some of Montana's beef operations with presentations on industry relevant topics by University Extension Beef Specialists and Researchers (see attached schedule). The committee felt this format might generate greater interest in the Beef Symposium and create an improved environment for speaker participate interaction. In addition, it would enable some of the leading cattle operations in Montana to showcase their operations, and provide their producer perspective to the symposium.

Drs. Glenn Duff and Rachel Endecott provided considerable leadership and effort in arranging the tour stops and symposium logistics.

There are 60 participants pre-registered for the 2013 WSAS Beef Symposium.

2013 WSAS Beef Symposium Committee

- Dr. John Hall, Univ. of Idaho, Chair
- Dr. Glenn Duff, Montana State Univ.
- Dr. Brett Kirch, Colorado State Univ.
- Dr. Dan Faulkner, Arizona State Univ.
- Dr. Dale Zobell, Utah State Univ.
- Dr. Reinaldo Cooke, Oregon State Univ.
- Dr. Scott Lake, Univ. of Wyoming

2013 Western Section Beef Symposium Program

Attendees should meet in the north parking lot of the Animal Bioscience Building (ABB)on the Montana State University campus.

Please arrive between 7:30 am and 7:45 am as the bus will leave promptly at 8:00 am. Depart ABB at 8:00 Wednesday June 19, 2013

8:30 to 10:30

Stop 1 - MZBar Ranch Belgrade, MT – Tom Milesnick

MZBar ranch is situated on 1400 acres just north of Belgrade, Montana. This cow/calf operation(as well as hay and grain for the ranch) has 2 spring creeks – Thompson Spring Creek and Benhart Spring Creek – in addition to 5 miles of the East Gallatin River, for the skilled fisherman. All 3 streams produce large, healthy wild brown and rainbow trout. Tom Milesnickwill provide information on trout stream habitat restoration and pasture rotation.

R. L. Endecott. Montana State University, Bozeman. – Supplementation during drought.

Andy J. Roberts, L. Alexander, T. Geary, R. Waterman, and M. MacNeil. USDA, ARS, Fort Keogh LARRL, Miles City, MT. – Multi-sire breeding, how many calves will each bull produce?

11:00 to 1:00

Stop 2 - Churchill Cattle Co. Manhattan, MT –Dale and Nancy Venhuizen

Churchill Cattle Company is owned and operated by Dale and Nancy Venhuizen and their four daughters near Manhattan, MT, where their goal is to design, produce, and market the very best quality Hereford genetics for the local, national, and international beef industry.

D.Kress. Montana State University, Bozeman-Heterosis: the forgotten free lunch

D. Faulkner and S. Lake. University of Arizona and University of Wyoming. Relationship of voluntary intake and cow survival under range conditions

2:00 to 3:30

Stop 3 –Red Bluff Ranch Norris, MT -Montana State University

Red Bluff Ranch is located near Norris in Madison County, Montana, along the west side of the Madison River. The operation comprises 13,750 acres of land, 10,000 deeded and 3,750 leased. Most of this land is rangeland, with limited hay meadows along the valley bottoms. Elevations range from 4,600 feet to 6,200 feet above the Madison River canyon. The ranch occupies most of the once thriving late 19th-early 20th century gold mining community in the Hot Springs Mining District which was second only in gold production to Alder Gulch. At its peak of activity, there may have been a population of approximately 3,000. The ranch nearly surrounds the town of Norris. The founder of Norris, Alexander Norris may have owned much or all of the Red Bluff Ranch at one time as The Red Bluff Research Ranch (previously known as the Rowe Brothers Ranch) was purchased for \$164,000 (\$16.83 per acre). The total acreage was 9,746. Two U. S. Forest Service Grazing Permits (Muddy Greek, Cache Creek) in the Gallatin National Forest came with the Rowe property. Some small additional land exchanges and purchases have taken place over the last 45 years. The grazing permits were returned to the Forest Service in 1976. A new lambing facility and mixing barn at

the ranch was constructed in about 1990. This made lambing much easier. There are currently about 170 head of cattle and 900 head of sheep maintained on a year round basis at the research ranch. These livestock along with the range areas are used for both teaching and research.

G. Brester. Montana State University, Bozeman. –Does the Cattle Cycle Still Exist?

4:00 to 5:00

Stop 4 – Bozeman Agriculture Research and Teaching (BART) farm Bozeman, MT

BART, formerly known as 'The Towne Farm' is located west of 19th Street and the main MSU campus. This farm comprises approximately 430 acres and houses the Oscar Thomas Nutrition Center, Miller Stock Pavilion, Equine Center, Horseshoeing School, Feed Mill, and Beef Center. The farm is dedicated to the service and support of research, teaching and extension activities relating to livestock and livestock management.

Date: June 21, 2013 Subject: 2013 WSASAS Awards Committee Report From: J. Bret Taylor, Awards Committee Chair To: President Glenn Duff and WSASAS membership

The Awards Committee is charged with receiving nominations, reviewing nomination materials, and selecting recipients for the Distinguished Service, Distinguished Teaching, Extension, and Young Scientist Awards. Committee membership consisted on S. Archibeque, J. Berardinelli, R. Endecott, S. Ivey, D. Rule, and J. Taylor (chair). The initial call for award nominations was posted in the January 2013 Secretary's Letter. The final call for award nominations was posted in the March 2013 President-Elect's Letter. The Awards Committee requested and the Executive Committee approved four \$50 awards to be presented to the nominators of the award recipients; this was advertised to the membership in the President-Elect's Letter. ASAS hosted a web-based nomination platform to receive all required documents from nominators. For the Distinguished Service, Distinguished Teaching, Extension, and Young Scientist Awards, 2, 3, 2, and 5 nominations were received,

respectively. The Awards Committee met (phone) April 24 for a brief training on how to navigate the web-based awards system. Committee members were allowed 1 week to review the nomination packets. On May 1, the Awards Committee met (phone) and unanimously selected the following award recipients: Distinguished Service, Patrick Hatfield (Montana State University); Distinguished Teaching, Kristen Johnson (Washington State University); Extension, Scott Lake (University of Wyoming); and Young Scientist, Kimberley Vonnahme (North Dakota State University). Respective recipients for the \$50-nominator awards were Glenn Duff, Margaret Benson, Doug Hixon, and Joel Caton. Award results were presented to the Executive Committee on May 9. From May 10 to 12, nominators of award recipients were notified; all other nominators were notified by e-mail of the results.

Applied Animal Science Award Report

June 13, 2013 Chair: Connie K Larson, Industry Director

Committee members (Sponsor recognition):

Court Campbell (Personal Sponsor) Mark Branine (Zinpro) Dan Dhuyvetter (Ridley Block) Kristy Dorton (Diamond V) Allison Grove (AG Research, LLC) Kim Hagar (CHS) Ben Holland (Merck) Mike Hubbert, NMSU Jim Killen (Personal sponsorship) Sonda Killen (Personal sponsorship Jeremy Martin (Great Plains Consulting) Trey Paterson (Padlock Ranches)) Steve Stafford (Personal sponsorship) Marshall Streeter (Merck) Kelcey Swyers (Ranchway Feeds) Gary Tibbetts (Zinpro)

Sponsorship for the award included both corporate and personal contributions from committee members. The total sum collected was \$1000.00, which was divided 50/30/20 for the monetary awards.

Thirteen papers (NMSU, 3; U of NE, 3; UW, 2; NDSU, 2; USDA, 2; and UC Davis, 1) were submitted for the award. Committee members reviewed the papers and provide their

rankings of the top three papers. The top three papers were as follows:

1st Place - EFFECTS OF POST-AI NUTRITION IN FERTILITY OF YEARLING BEEF HEIFRS; R. P.

Arias^{*1}, P. J. Gunn², R. P. Lemenager², G. A. Perry³, G. A. Bridges⁴, and S. L. Lake¹, ¹University of Wyoming, Laramie, WY, ²Purdue University, West Lafayette, IN, ³South Dakota State University, Brookings, SD, ⁴University of Minnesota, St. Paul, MN.

2nd Place - EFFECT OF PUBERTAL STATUS AND NUMBER OF ESTROUS CYCLES PRIOR TO THE BREEDING SEASON ON PREGNANCY RATE IN BEEF HEIFERS; R. A. Vraspir^{*1}, A. F. Summers¹, A. J. Roberts², and R. N. Funston¹, ¹University of Nebraska, West Central Research and Extension Center, North Platte, NE, ²USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT.

3rd Place - EFFECTS OF MATERNAL NUTRITION AND RUMEN-PROTECTED ARGININE SUPPLEMENTATION ON EWE AND POSTNATAL LAMB PERFORMANCE; J. L. Peine^{*1}, G. Q. Jia¹, M. L. Van Emon², T. L. Neville¹, J. D. Kirsch¹, C. J. Hammer¹, S. T. O'Rourke³, L. P Reynolds¹, and J. S. Caton¹, ¹Departments of Animal Sciences and ²Pharmaceutical Sciences, North Dakota State University, Fargo, ND and ³Hettinger Research Extension Center, Hettinger, ND.

Young Scholar Recognition Program (YSRP) Committee Report

June 19, 2013

Chair: Connie Larson, Zinpro

Members: Pat Hatfield*, MSU Ryan Ashley*, NMSU Jack Whittier*, CSU Kelsey Quinn*, Sr. Graduate Representative James Graves *, Jr. Graduate Representative Gary Moss, U of WY Greg Lardy, NDSU Michael MacNeil, Montana * Members in attendance at the meeting

The committee met to discuss the YSRP process and program format for the invited talks of recipients. For 2013, there were four nominees in M.S. degree category (two from New Mexico State University and two from Montana State University), and no nominees in the Ph.D. degree category. The Young Scholar Recognition recipients were Kate Sharon, MSU, and Melanie Beckman, NMSU.

In view of the lack of Ph.D. nominees, the committee discussed the Western Section as having a greater number of M.S. graduate students compared to those pursuing a Ph.D. It was decided to revise the YSRP to include one Ph.D. recipient and two M.S. recipients. The group also considered the monetary award and has suggested that the Ph.D. recipient receive a larger monetary award (\$600.00) and the M.S. recipients each receive a smaller amount (\$400.00). Before finalizing the decision, an inquiry will be made as to how these amounts line up with some of the other awards. With the revision in number of YSRP to be recognized, the time commitment for future meetings will need to allow for three 30 minute presentation.

The time designated for the invited talks was between the conclusion of the Sheep and Beef Symposias and prior to the Opening Reception. It was the feeling of the Committee that the light attendance may have been due lack of knowledge about the program since this is the initial year and scheduling. Many attendees were ready for a break before the start of the Reception. Overall, the committee thought that the recipients did a nice job presenting their research.

The committee would like to recommend that the hosting institutions give some consideration to the following options for the invited talks. First option would be to incorporate the talks into the schedule with the Competition Papers. This of course would depend on the number of Competition Papers need to be schedule. The other approach discussed was much like the approach that the Midwest Section has. The invited talks are incorporated into the presentation topic sections that fit with the recipients' research area. A third idea was a combination possibility that the Ph.D. recipient be included with the Competition Paper schedule and the M.S. recipients scheduled in topic sections. The committee felt that there needed to be flexibility allowing the host institution options for incorporating the invited talks into the program.

The suggested calendar dates were discussed among the members. These suggested dates are as follows:

Aug 30	Award site on-line ready to accept
	nominations
Sept through Dec	Monthly reminders for award
	nomination submissions
Jan 6	YSRP nominations due
Jan 8	Nominations out to the committee for
	review
Jan 29	Reviews completed and winners notified
Mar 5	Submission of Invited Proceedings Papers

The discussion regarding dates started with the concern that the Jan 6 deadline date for submissions would likely fall during a time that many Universities were between semesters. The committee agreed that the Invited Papers did not need to be submitted as early as the other abstract/ proceedings paper submission deadline since they did not require review and acceptance. It was a consensus that the early dates were not necessary and a revised time line was determined as follows:

YSRP nominations due
Nominations out to the committee for
review
Reviews completed and winners
notified (two week review period
acceptable to committee members)
Submission of Invited Proceedings
Papers

Committee members then discussed the nomination process and the challenges that were present during this first year. The challenges included lack of information on the website for the YSRP, poor communication in regards to the nomination process and expectations for both the abstract and essay, and required recommendation letters from Department Heads. The Committee would like to see the website information improved as well as more "user friendly" access to the information. Ideas that were generated in the discussion are as follows:

- 1. Tabs that can easily take you to the YSRP information.
- 2. Including a link to the YSRP information included in the "reminder emails" regarding requests for award nominations.
- 3. List of important dates.
- 4. Photos and bios of the current year award winners in order to bring a more personal touch to the information and to highlight the recipients from the previous year. Also include instant access to the Invited Proceedings Papers
- 5. More detailed communication on the expectations for each component of the nomination package.
- 6. Scoring guide.

There had been confusion with the process during this first year as to whether the nominator completed all components of the nomination process or whether it was a combined effort with the student. The committee agreed that it should be a combined effort and more specific directions were needed. The three components of the nomination process that generated discussion among the committee included nomination letters, essay and the abstract. The committee agreed to revise these three components.

- 1. Nomination letters. One letter to be submitted by the nominator and a second letter of recommendation to be determined by the nominator. The second letter is no longer required to be from the Department Head.
- 2. Essay. The essay will be more of a personal statement written by the student.
- 3. Abstract. The abstract will be more of an extended abstract that reflects the contents of the Invited Proceedings Paper.

To improve directions and information regarding the YSRP committee members agreed to work on assignments. Kelsey Quinn and James Graves will work on providing the guidelines for the essay. Pat Hatfield and Connie Larson will determine the guidelines for the extended abstract. Once these two components are completed and all committee members are in agreement, Jack Whittier and Ryan Ashley will evaluate the scoring guidelines and make any necessary adjustments.

2013 WSASAS Graduate Student Paper Competition Minutes Submitted by: Chad J Mueller, 2013 Chair (Oregon State University)

The 2013 WSASAS graduate student paper competition was held on Thursday, June 20, 2013 at the Museum of the Rockies in Bozeman, MT. This year's committee members included: Chad Mueller (OSU; chairman), Kraig Peel (CSU), Scott Lake (UW), Eric Scholljegerdes (NMSU), Ryan Ashley (NMSU), Chris Schauer (NDSU), and Michelle Mousel (USDA-Dubois). There were 21 paper presentations from 8 universities. Institutions included: Colorado State University – 4; Kansas State University – 1; Montana State University – 3; New Mexico State University – 4; North Dakota State University – 2; Oregon State University – 1; University of Wyoming – 4, and University of Nebraska – 2. The 2014 competition will be chaired by Ryan Ashley (NMSU). No 'institutional' winners were announced for this year.

The 2013 winners were:

1st) Jena L. Peine, North Dakota State University

Effects of maternal nutrition and rumen-protected arginine supplementation on ewe and postnatal lamb performance. J. L. Peine^{1,*}, G. Q. Jia¹, M. L. Van Emon¹, T. L. Neville¹, J. D. Kirsch¹, C. J. Hammer¹, S. T. O'Rourke², L. P. Reynolds ¹, J. S. Caton, ¹Animal Sciences, ²Pharmaceutical Sciences, North Dakota State University, Fargo, United States.

2nd) Kelsey E. Quinn, New Mexico State University

Regulation of vascular endothelial growth factor (vegf) by (c-x-c motif) ligand 12 (cxcl12) in ovine caruncle explants. K. E. Quinn^{1,*}, R. L. Ashley¹, ¹Animal and Range Science, New Mexico State University, Las Cruces, United States.

3rd) Chris L. Shelley, New Mexico State University

Effect of cobalt supplementation on rumen fermentation and blood metabolites.

C. L. Shelley^{1,*}, L. N. Trace¹, A. L. Salazar¹, K. Marchetti¹, L. Schmitz¹, E. J. Scholljegerdes¹, C. K. Larson², S. Ivey¹, ¹Animal and Range Sciences, New Mexico State University, Las Cruces, NM, ²Zinpro Corporation, Eden Prairie, MN, United States. Suggestions:

The committee recommends for the 2014 competition to reinstate the 'Institution' winner award. We acknowledge the transfer of award funds for the "Young Scholar Award", which is commended, but committee members would be willing to solicit some Industry funds for a small award (or traveling trophy). [During the 2013 business meeting Dr. Shanna Ivey commented that in the previous year's business meeting that Institutional winners would still be recognized and added to a 'traveling trophy'. These comments were not added to the final minutes in 2012, therefore were not available for the current committee's final decision. It was determined during the 2013 business meeting that Zinpro would sponsor a traveling trophy and that the 2013 Institutional winner is New Mexico State University.] CJM

The committee has suggestions for improvement of the abstract/proceedings submission process. We recommend that the online system lock students out of all sections once "Graduate Competition" is selected. The current system will not provide the chairman access to proceeding papers that have been submitted after the deadline, therefore this needs to be corrected for 2014.

Western Section American Society of Animal Science Proceedings Publication Committee Report and Recommendation

November 11, 2012 Presented by Dennis Hallford

At the request of the WSASAS Executive Committee, the Proceedings Publication Committee met via teleconference on Friday, November 9, 2012 at 1000 (Central Time). Committee Members present were Meghan Wulster-Radcliffe (ASAS Chief Executive Officer), Chris Schauer (North Dakota State University), Bret Taylor (USDA ARS), Jack Whittier (Colorado State University), and Dennis Hallford (New Mexico State University), Chair). Also in attendance were Jacelyn Hemmelgran (ASAS Chief Operations Officer) and Jennifer Gavel (Program Director). The meeting agenda included the following 4 items:

- Description of the B-Com System for Abstract submission and possibly WSASAS Proceedings submission – Meghan, Jacelyn, Jennifer
- 2. Discussion of the purpose of the WSASAS Proceedings papers - preliminary dissemination of research results, vehicle for introducing students to scientific writing, refereed journal article, etc. – All Committee Members
- Based on the purpose of the Proceedings, discussion of most the appropriate method of disseminating the information in the Proceedings – traditional method (B-Com System, outside printer) vs Manuscript Central – All Committee Members
- 4. If a consensus is reached, formulation of a recommendation(s) for submission to the Western Section Executive Committee

The meeting began with Meghan describing the process of publishing the 2012 WSASAS Proceedings after FASS declined to be involved. After the ASAS meeting in Phoenix, ASAS staff members examined a number of other options to process abstracts for subsequent meetings and concluded that B-Com Event Technologies offered a number of services that appear to be superior to those used previously including the ability to process the WSASAS Proceedings in a manner essentially identical to the traditional method. The second option for handling the Proceedings is submission through Manuscript Central. The attachment summarizes Meghan's thoughts on advantages and disadvantages of the 2 options. Committee consideration of these options involved a number of issues including: revenue generated for WSASAS by publishing the Proceedings, current method for review of manuscripts (authors submit a form stating that manuscripts have been reviewed in-house), review of Graduate Student Competition papers (usually extensively reviewed by faculty before submission), submission through Manuscript Central offers the attractive advantage of allowing Proceedings papers to be searched on-line, effects of the Manuscript Central review system on future ability to publish results in another refereed journal (see attached), and the possibility of extensive submissions by authors who do not attend the meeting if the Proceedings are perceived to be "refereed publications."

Based on these discussions, the Proceedings Publication Committee recommends that the 2013 WSASAS Proceedings be submitted and published through the B-Com system and that review of manuscripts be handled by methods used previously at member institutions. The Committee further recommends that when the WSASAS Meeting is held in conjunction with the ASAS Annual Meeting, the ASAS staff be allowed the flexibility to manage submission and publication of the Proceedings in a manner compatible with abstract submission for the Joint Annual Meeting.

DISTINGUISHED SERVICE AWARD

Proceedings, Western Section, American Society of Animal Science

Vol. 65, 2014

Putting ruminants back into crop farming: Systems level thinking and production for the future

Patrick G. Hatfield

Department of Animal and Range Sciences, Montana State University, Bozeman, MT 59717

Abstract: This paper is not a call to return to 1940's farming. Nor is it an endorsement of organic agriculture or a condemnation of confinement finishing of beef and sheep on grain diets. Rather, it is testimony that we as a nation cannot continue with some types of grain production systems due to environmental and eventually, profitability issues. There are solutions to sustainability of crop farming in the U.S. that maintain soil quality, reduce erosion and non-point pollution, and maintain true market profitability while providing food for a growing world population. Environmental sustainability and farm profitability may be improved by putting grazing ruminants back into farming systems. This would provide high quality forages for sheep and cattle and protein for an increasingly affluent populace.

INTRODUCTION

It is estimated that by 2050 there will be 9.6 billion people on Earth (UN, 2014) and they will be more urbanized moving water away from agricultural use. At the same time per capita income will continue to grow in many parts of the world resulting in an increased demand for food particularly meat products (APLU, 2010). Increasing world populations put greater demands on environmental services including food production and clean water and are fueling debate on how we produce and distribute food. Often it is argued that we need to apply incremental (i.e. another bushel/ acre) solutions to current production methods that will allow business as usual, but on a bigger, and hopefully more efficient, scale. We cannot continue, however, to apply or increase many of our current agricultural practices without compromising future generations' ability to feed themselves. This paper addresses the problem of how to meet increasing demands for food, especially protein, without compromising production or environmental sustainability.

Population and food

Eight hundred forty-two million (or 1 out of 8) people in the world today are undernourished; however, food supply to the world's population is not limiting (WFP, 2014b). High estimates of food wasted (39 million metric tons or 40 to 50% annually in the U.S.; Slivka, 2012; EPA, 2014) and recreational consumption of empty calories leading to obesity (particularly in developed countries but also worldwide) suggest that worldwide food production exceeds demand. The World Food Programme (2014a) suggests that 3 of the main causes of hunger are poverty, lack of investment in agriculture, and food wastage rather than a shortage of food production. Therefore, agriculture may not need to continue to produce more and more food at the expense of our agricultural lands simply to fuel a society's insatiable food consumption leading not only to food waste, but an epidemic of poor health resulting from over consumption. When sustainable consumption is partnered with programs that address poverty and the resulting problems associated with food distribution, sustainable agriculture can be a partner in feeding an increasing affluent and world population.

Current farming practices and sustainability

As much as the lack of understanding of agriculture exists by urban dwellers, the understanding of grazing ruminants by crop producers and vice-versa is often limited. Specialization that has led to the decoupling of integrated plant and animal agriculture is not only an industry issue but our grant funded academic focus on basic science has often limited our ability to look at whole systems agricultural issues. As defined by the USDA as set forth in U.S. Code Title 7, section 3103 is: "The term "sustainable agriculture means an integrated system of plant and animal production practices having a site-specific application that will over the long-term:

- Satisfy human food and fiber needs.
- Enhance environmental quality and the natural resource base upon which the agriculture economy depends.
- Make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls.
- Sustain the economic viability of farm operations.
- Enhance the quality of life for farmers and society as a whole.

Specialization. Since the 1940's crop diversity has generally declined in North America (Brummer, 1998). Commodities per farm decreased from an average of 5 in 1900, to 3 in 1970 and just over 1 in 2002 (Dimitri et al., 2005). Since the 1970's, U.S. farm policy has focused on increasing rather than controlling production making a

favorable financial environment for monoculture production. This focus on decreasing costs by increasing production (i.e. economy of scale) has shifted crop and livestock production from on-farm integration to spatially separated and specialized operations (Russelle et al., 2007), decoupling crop and livestock production (Brummer, 1998; Sanderson et al., 2013). In recent years, the use of corn for ethanol production has further contributed to increased specialization and acreage used for growing crops for energy production has competed with acreage used growing crops for food production. The number of hectares dedicated to corn production has risen dramatically since 1993 (Iowa Corn Promotion Board, 2014) and in 2013 ethanol production from corn accounted for 27% of corn usage compared to 11% for exports and 9% for beef production (Iowa Corn Promotion Board, 2014).

Other factors contributing to increased specialization are government farm subsidy programs, crop insurance limitations to grazing or having cover crops, increased reliance on petroleum-based products, and improved technology. Many economists and agricultural scientists consider the U.S. farm policy program as being counter to improving environmental sustainability (Ostria, 2013). This is not only a government and industry issue. Our predominant academic focus on basic science has limited our ability to look at whole systems agricultural issues. There is growing evidence that these specialized, low diversity production systems do not produce optimal outcomes for the long-term profitability and sustainability of agriculture and the environment (Hesterman and Thorburn, 1994; Karlen et al., 1994; McIsaac et al., 2001; Trainer et al., 2005). Most crops use only 30 to 50% of the fertilizer applied with the remainder moving into either water systems or the atmosphere (Tilman et al., 2002). Under current production practices, corn acreage in high rainfall and irrigated systems typically loses nitrogen to water at annual rates of 20-40 kg/ha. Of the 37.9 million ha of corn planted in 2007, it is estimated that 117 million kg of nitrogen were deposited into national waterways (Simpson et al., 2008). In addition, the Ogallala Aquifer supports nearly one-fifth of the wheat, corn, cotton and cattle produced in the United States; however, withdrawals currently exceed the natural recharge of the aquifer which is unsustainable (NRCS, 2012). Furthermore, when food is wasted – the water used to grow that food is also wasted (WFP, 2014a). These data suggest that conventional corn production, in part driven by farm subsidy programs, cannot be sustained in a manner that allows future generations of Americans to meet their food needs from American farm land.

Soil erosion. Twelve hundred hectares of productive farmland are lost to development each day in the U.S. (EPA, 2013); however, a bigger issue may be loss of farmable land due to erosion. During the last 40 years, nearly one-third of the world's arable land has been lost to erosion and continues to be lost at a rate of more than 10 million ha/yr (Pimentel et al., 1995). Soil erosion is a major environmental threat to the sustainability and productive capacity of agriculture (Pimentel et al., 1995). We typical think of these issues only impacting less developed nations;

however, the NRCS reported that 30% of farmland in the U.S. has been abandoned due to erosion, waterlogging, and salinization (NRCS, 2014). The report goes on to estimate that in the U.S. we lose 2.7 kg of farmable soil for each 0.45 kg of food consumed compared to 8.1 kg of farmable soil per 0.45 kg of food consumed in China. It can take up to 500 years to build 2.54 cm of topsoil and growing good crops requires about 15.24 cm. As Duffy, an Iowa Extension Economist, concluded in 2012, "the value of the lost soil to the land owner may not be great; however, it is measureable and will have an impact over time. Soil for the land owner is a bit like the story of removing bricks from a wall: you can remove the bricks one at a time without any trouble until you remove one too many and the wall collapses. A land owner can tolerate soil erosion a little at a time, but at some point it is going to cost, and they won't know what they've got until its gone". President Franklin D. Roosevelt also stated that "A nation that destroys its soil destroys itself" (NRCS, 2014). Soil erosion on U.S. cropland decreased 43% between 1992 and 2007 (NRCS, 2007); however, NRCS estimates of average soil losses may not reflect true detrimental soil losses (Cox et al., 2011). For example, on some acreages in Iowa, single storm events with high rain fall triggered soil losses 12 times greater than NRCS estimates (26.8 vs. 2.3 metric tons/ ha annually; NRCS, 2007; Cox et al., 2011). These levels of soil losses are not sustainable and could be prevented. Although we cannot "take back" land lost to development or water "mined" from underground reserves, we can look at integrated systems that would drastically reduce soil and nutrient loss from farm lands while providing high quality foraging opportunities for sheep and cattle producers.

Increased diversification of cropping systems with cover and perennial crops

Economy of scale and specialization allow for decreased costs through increased production; however, diversification in agricultural production also has economic benefits related to risk management and economy of scope (Sanderson et al., 2013). Economy of scope refers to situations where the cost of producing multiple outputs is lower for a single, integrated farming system than for multiple specialized farms. This can occur when growing crops in rotation increases yields or reduces inputs relative to growing individual crops continuously in monoculture. Davis et al. (2012) conducted a 9-yr study in Iowa that compared a conventionally managed 2-yr rotation (maize-soybean) that received fertilizers and herbicides with two diversified cropping systems: a 3-yr rotation (maize-soybean-small grain + red clover) and a 4-yr rotation (maize-soybean-small grain + alfalfa-alfalfa) managed with lower synthetic N fertilizer and herbicide inputs and periodic applications of cattle manure. Results showed that grain yields, mass of harvested products, and profit in the more diversified systems were similar to, or greater than, those in the conventional 2-yr rotation system, despite reductions of agrichemical inputs (Davis et al., 2012). Weeds were suppressed effectively in all systems, but freshwater toxicity of the more diversified systems was two orders of magnitude lower than in the conventional system. Results of this study indicate that more diversified cropping systems can use small amounts of synthetic agrichemical inputs as powerful tools with which to tune, rather than drive, agro-ecosystem performance, while meeting or exceeding the performance of less diverse systems with less negative environmental impacts.

Numerous articles have that documented the functional value of cover crops to improve ecosystem services and environmental quality across a wide range of growing conditions (Sanderson et al., 2013). Among these outcomes, cover crops reduced soil erosion by protecting soil from raindrop impact, improving soil structure and increasing infiltration. Cover crops can enhance soil biological abundance and activity, thereby contributing to increased N mineralization rates and N supplying potential of soil. Farmers in Australia are replacing annual pastures with perennial pastures providing a range of benefits including: improved hydrological balance to reduce dryland salinity, subsoil acidification and water-logging; additional management tools for herbicide-resistant or problem weeds, and improved soil nutrient and carbon stocks (Bell et al., 2013).

Increase diversification by incorporating livestock in farming systems

Another example of economy of scope is when grain or forages from crop production are used for animal feed, and livestock manure is used for fertilizer, reducing costs for crop and livestock production on a single farm. Livestock incorporated into a diversified crop farming system has the potential of synergist benefits for both the crop and the livestock producer (Sanderson et al., 2013); however, crop residues are an underutilized feed resource for ruminants in the U.S. The American Forage and Grassland Council estimated that only 25% of crop residues are used each year. This would provide feed energy for 11.4 million beef cows; however, if 100% of crop residues were utilized they would supply the energy requirements for 45.5 million cows, essentially the entire U.S. beef herd in the peak year of 1975 (as cited by Gill, 2014). Realize this estimate does not consider quality or caloric density, but it does raise the point of under-utilized feed resources that have a potential for greater inclusion in ruminant feeding systems.

Research has shown that economy of scope in agriculture can be significant. Crops and livestock were both produced at a lower costs for integrated compared to specialized farms in Wisconsin and the cost of joint crop and livestock production averaged 14% lower compared to costs for specialized crop or livestock production in Missouri (Sanderson et al., 2013). In these studies, total cost efficiency tended to be lower for the integrated farms because they were less efficient than specialized farms at adjusting the mix of inputs used in production to minimize costs. Other researchers have documented that cover crops can improve profitability (Sanderson et al., 2013).

Dual purpose cereal and canola for forage during the vegetative stage and harvesting for grain is practiced throughout southern Australia (Bell et al., 2013). Cereal-ley systems involving annual self-regenerating pasture legumes in crop rotations have improved soil fertility, increased cereal yields and livestock production in southern Australia since the 1930s (Puckridge and French, 1983). Sacrificially grazing crops when expected grain yield is low and/or livestock prices are attractive relative to grain provides further flexibility in crop-livestock management system (Bell et al., 2013). This type of diversification has increased livestock productivity by filling feed gaps and increased both livestock and crop productivity by 25 to 75% (Bell et al., 2013).

At Montana State University our goal has been to develop integrated crop/livestock production systems that are economically and environmentally sustainable, provide benefits to both grazing livestock and crop farming systems, and offer opportunities for rural development through new enterprises focused on targeted grazing. We have evaluated several interconnected issues from a systems level approach soil health and nutrient cycling, production of including: greenhouse gases, residue management, pest management, crop yields and profitability. Our research has shown a number of benefits (Goosev et al., 2004, 2005; Hatfield et al., 2007a,b,c; Sainju et al., 2010, 2011). First, it helped reduce tillage intensity and soil erosion, promoted nutrient cycling, and enhanced soil tilth. Second, sheep grazing stubble resulted in the greatest levels of mortality in the wheat stem sawfly (one of the most damaging insect pests to wheat production in the U.S.) compared to burned, tilled, and no-till systems. Third, sheep grazing of alfalfa aftermath and early spring growth reduced alfalfa weevil numbers without reducing subsequent hay production. Fourth, in a 4-yr comparison of winter and spring wheat rotations with summer fallow, targeted sheep grazing did not reduce yields when compared to tilled- or chemical-fallow (Lennsen et al., 2013). Finally, in current work comparing tillage, herbicide use, and grazing as methods of terminating cover crops we observed that grazing generated a quality lamb product, out of the typical season of lamb availability, while providing the least cost method for cover crop termination.

Opposition to incorporating livestock into farming systems

While there are many advantages to incorporating livestock into farming systems, there are issues related to forage finishing of beef and sheep. The 4 main arguments against it are:

- 1. Seasonal availability of high quality forage in sufficient quality, quantity and duration to finish, rather than simply grow, the animal.
- 2. Competition between finishing operations and breeding cows and ewes.
- 3. Flavor and quality of forage finished beef and lamb does not meet typical consumer preference.
- 4. Weight gains on forages are not as great as gains on high grain diets.

Forage provided by perennial and cover crops in diversified farming systems provides an additional source of forage for livestock at different times of the year and helps address the first 2 arguments against forage finishing. Finishing beef and sheep on cover crops may not be a replacement to confinement finishing, but provide methods to reduce reliance on grain for producing a quality meat product.

Currently there are 34 million ha of corn grain production in the U.S (NASS, 2013). If a 4-yr crop rotation is implemented by taking out 27% of corn production that goes to ethanol, then 21 million acres could be planted to some type of cover crop annually. Assuming a conservation value of 0.9 metric tons/ha of forage produced and a feed conversion of 3.1 kg feed/0.45 kg gain (Hatfield, unpublished data), 5.4 billion extra kg of lamb or beef could be produced annually. If soybean acreages are also included in the rotation, at an estimate of 1.8 metric tons/ha, then the extra kg of lamb produced is easily tripled.

Lambs in the intermountain west are typically marketed in the fall of the year; however, fall marketing does not provide a year-around supply of quality lamb. Barley stubble and other crop residues are inexpensive winter feed resources (Hatfield et al., 1999) and grazing lambs on them would allow alternative marketing times. Nichols et al. (1992) reported that 99% of the lambs held over the winter on stubble fields graded choice after subsequent confinement feeding. Finishing lambs on cover crops (typically in late June in the Northern Great Plains) would allow marketing of lamb at other times. These scenarios may involve finishing lambs at different ages; however, carcass characteristics of lambs have sometimes (Carpenter et al., 1965; Batcher et al., 1969), but not always (Field and Whipple, 1998; Jeremiah et al., 1998; Hatfield et al., 2000) been negatively affected by age.

Some researchers have reported no differences in aroma, flavor, juiciness, connective tissue or tenderness among steers finished on pasture, in the feedlot, or a combination of the two systems (Lagreca and Pordomingo et al., 2009); however, there is no doubt that most of the time, grass finished beef do not provide a consistent quality product that affluent consumers desire. On the other hand, finishing lambs on grass typically results in a more favorable finished product than beef. Blackburn et al. (1991) concluded that meat from lambs raised on forage diets versus ad libitum concentrates contained less fat. Forage finishing systems may improve production efficiency and processing by preventing excessively fat carcasses from lambs slaughtered at 47 kg of live weight (Jacques et al., 2011). It appears that in lambs forage-based diets have the potential to produce similar muscle development and a leaner product for consumers. Forage-based growing systems that take advantage of cover crop termination, enterprise decisions on grazing vs leaving a grain crop for harvest, and harvest and feeding (or swath grazing) of perennial forage crops may be an alternative to confinement-fed lamb production as they utilize natural resources and provide a high-quality meat desired by consumers (Grunert et al., 2004).

Beef do not typically gain as well on forage diets as on grain diets. Cattle usually spend 3 to 6 mo in a feedlot and average 1.1 to 1.8 kg ADG (NCBA, 2006). A 1.36 kg/d target is common for pen-fed cattle finishing a diet which is nearly

impossible to achieve on grass. Producer testimony from NZ reports weight gains in this range by cattle consuming high sugar grasses (Pure Advantage, 2014); however, most information indicates reliable gains on grass in the 1 kg range. These high sugar grasses, however, could be incorporated into cover crop mixes to increase BW gain. On the other hand, Pordomingo (2006) suggests that this level of growth performance may not always be needed to produce well finished grass-fed beef. Meat quality and consistency has been adversely affected by periods of low (< 0.45 kg/d) gains; however, either a steady life-time gain or gain in the last 100 d above 1kg/d can lead to a product with desirable eating quality. The right genetics combined with good nutrition starting with a well nursed calf insures adequate adipose cell development setting the stage for proper finishing later in life. The potential for using cover crops to shorten the confinement feeding period and improve crop land via incorporation of cover crops may have cost savings benefits to beef producers and environmental benefits to corn farmers. Systems could be adapted that use some amount for grain finishing either in confinement or in conjunction with farming systems (i.e. "feedlots" on crop ground) to match with seasonal forage quality and availability as well as market demands.

The majority of research has also reported that lambs grow faster on concentrate-based diets than on forage-based diets (McClure et al., 1994, 2000; Murphy et al., 1994; Fimbres et al., 2002; Turner et al., 2002; Borton et al., 2005; Demirel et al., 2006; Archimède et al., 2008; Jacques et al., 2011). Ad libitum consumption of concentrates results in fatter lambs compared to those fed forage diets when the lambs are slaughtered at a constant final weight (Fisher et al., 2000; Archimède et al., 2008: Resconi et al., 2009). Some researchers, however, have shown that high-quality pastures and forages can yield similar ADG to what is achieved in drylot and produce competitive high-quality lamb carcasses (McClure et al., 1994; Aurousseau et al., 2007). Grovum (1988) suggested that sheep consumed the most energy when fed diets containing relatively large amounts of alfalfa compared with concentrates. Hatfield et al. (1999) reported that ewes, bred to lamb in May, grazed yeararound and supplemented with a 25% CP supplement from December until March, did not differ in kg of lamb weaned per ewe when compared to a more intensive system in which ewes were confinement fed for 6 mo on high quality alfalfa and grain supplements. In current research at MSU, Hatfield et al. (unpublished data) is observing lamb weight gains in the range 0.27 kg/d for lambs finished on a high barley diet compared to 0.27 kg/d for lambs finished on a high alfalfa diet. Furthermore, finishing lambs on alfalfa has lower potential for digestive upset compared to grain finishing making it easier for any sheep producer to finish lambs on the farm.

An additional point to be made is that absolute highest gains in a single system enterprise may not always be the driving force in determining profitability. Consider when livestock and farming enterprises are combined in the field. In the first year of a study at MSU we compared three methods of terminating a cover crop. These were tillage, herbicide, and grazing lambs for slaughter. Based on current lamb prices the grazed plots returned \$283/ha and resulted in ground ready for seeding with no additional tillage. The herbicide and tilled plots required approximately \$14/ha for termination and seed bed preparation. Forage-based diets may offer the option of reduced daily production costs in comparison to confinement systems (Notter et al., 1991; Woodward and Fernández, 1999).

Another consideration is what value "moving the feedlot" to the field can have on farmland organic matter, soil microbiome health, and overall soil health. Observations in Argentina by Pordomingo (2006) of a 17,000 beef finishing operation on farmed ground indicates the potential to add significant organic matter to farmed soils. J. Kirkegaard (CSIRO, personal communication) has made similar observations in sheep. Grazing of stubble and crops by sheep may make more N available to crops which could increase yields. This could be a function of converting forage to indigestible OM mixed with N containing microbial cell fragments from the sheep digestive system which is returned to the soil. In ongoing research at MSU we are exploring the impact on soil health of finishing lambs on wheat stubble, being fed either a high grain or high alfalfa diet. What impact this has on the crop farming system is yet to be determined, but we are hopeful that this type of finishing system can help improve soil health and help reduce off farm inputs into crop production systems.

CONCLUSION

Slow expansion of innovative farming systems in the United States is as much a policy and market problem as a science and technology problem (Reganold et al., 2011). Although only a third of U.S. farmers receive commodity or conservation payments under the Farm Bill which, it has a major influence on what, where, and how food is produced with most elements of the Farm Bill not designed to promote sustainability (Reganold et al., 2011). In addition, most USDA funded research programs fund research focused on incremental increases in conventional agricultural production rather than systems level research leading toward transformative agricultural systems. Integrated crop-livestock research projects are large, not only in land area and livestock requirements, but also in financial commitment by research agencies. These projects are often multi-disciplinary in nature requiring compromise and cooperation among scientist to ensure that adequate data are collected (Tanaka et al., 2008).

The objective of this paper is not to criticize confinement finishing of lamb and beef; however, with concerns over the sustainability of corn production in this country, the opportunity to reduce the reliance of ruminants on corn via incorporation of grazed and harvested cover crops into farming systems has the potential to improve sustainability of crop production while providing for an increasing demand for meat and food. In our extension efforts to promote diverse integrated systems that incorporate sheep and cattle in to farming systems in Montana, we do not advocate a return to 1940's family farming, rather enterprise level integrated systems. The level of education and expertise to be competitive within a singular farming enterprise is monumental. However, enterprise level partnerships either within a family (one sibling the farmer, the other the sheep producer), corporation, or among totally separate business entities allow for specialization and focused expertise by individuals, but systems level farming/grazing to promote sustainability.

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Genome assembly of the putative etiologic agent of epizootic bovine abortion¹

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ABSTRACT: Epizootic bovine abortion (EBA), commonly known as "foothill abortion", is the leading cause of beef cattle abortion in California, responsible for the loss of an estimated 45,000 to 90,000 calves per year. Disease incidences for EBA have been reported in California, Nevada, and Oregon. In the 1970's, the soft-shelled tick Ornithodoros coriaceus, or "pajaroello" tick, was confirmed as the vector that transmits the disease. In 2005, a novel deltaproteobacterium was discovered as the etiologic agent of EBA (aoEBA). It is not possible to grow this organism in culture using traditional microbiological techniques; rather it can only be grown in experimentally-infected immunodeficient mice. This led to the development of a live bacterial vaccine consisting of a quantifiable number of aoEBA-infected mouse spleen cells. Difficulties and costs associated with production of this live bacterial vaccine motivated our investigation into the development of a recombinant vaccine as an alternative approach to help prevent EBA. The experimental objectives of this study were to assemble a reference genome for the novel aoEBA deltaproteobacterium, and identify highly transcribed bacterial genes encoding potential antigenic proteins as candidates for the development of a recombinant vaccine. DNA and RNA were extracted from spleen tissue collected from experimentally-infected immunodeficient mice 68 days following their exposure to the aoEBA deltaproteobacterium. This combination of mouse and bacterial DNA were sequenced and aligned to the mouse genome. Mouse sequences were then subtracted from the aoEBA genome and the remaining sequences were de novo assembled at 50X coverage into a 1.82 Mbp complete closed circular deltaproteobacterial genome, containing 2,250 putative protein coding sequences. The most closely related pathogen to aoEBA, Lawsonia intracellularis, contains 1.72 Mbp of genomic DNA. This suggests that the 1.82 Mbp of assembled aoEBA DNA is likely to represent the entire deltaproteobacterial genome. RNA was reverse transcribed and likewise sequenced and aligned to the murine genome. Sequences remaining after removal of the murine data represent genes in the aoEBA

genome that are being expressed during infection. Highly expressed protein coding sequences, discovered through whole transcriptome shotgun sequencing, represent potential antigenic candidates for the development of a recombinant vaccine. The assembly of the complete circular aoEBA genome and discovery of highly expressed proteins through RNA sequencing provides the basic background information required to further the development of a recombinant vaccine for California's leading cause of abortion in beef cattle.

Key words: foothill abortion, genome assembly, recombinant vaccine

INTRODUCTION

Epizootic bovine abortion (EBA), commonly known as "foothill abortion", has been a persistent problem in the beef cattle industry for more than 60 years (Howarth et al., 1956). Howarth et al. (1956) first defined EBA as a specific fetal syndrome with distinctive fetal pathology characterized by late-term abortion or birth of weak calves. In the 1970's the soft-shelled tick *Ornithodoros coriaceus*, referred to as the pajaroello tick, was identified as having the same geographic distribution as EBA, and was confirmed to be the vector transmitting EBA (Schmidtmann et al., 1976). Although the tick appears to be spreading due to the increased eastward movement of cattle to neighboring states, to date incidences have only been reported in California, Nevada, and Oregon (Teglas et al., 2006).

Diagnosis of EBA has typically been based on the presence of characteristic gross and microscopic lesions of the aborted fetus at necropsy, increased fetal serum immunoglobulin (Ig) concentrations, a history of exposure to the tick vector, and the elimination of other causes of abortion (Kennedy et al., 1983). The inability to experimentally propagate the causative agent of EBA led to many failed attempts of incriminating the causative microbe. The use of suppression hybridization PCR (shPCR) ultimately identified the causative agent of EBA as a novel deltaproteobacterium most closely related to organisms in the order Myxococcales (King et al., 2005).

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Identification of the putative etiologic agent of EBA (aoEBA) allowed for the development of new diagnostic methodologies. The visualization of aoEBA through immunohistochemistry (IHC) and DNA-based diagnostic techniques using polymerase chain reaction (PCR) have increased the sensitivity of diagnosis (Anderson et al., 2006; Brooks et al., 2011). The inability to culture this bacterium in media necessitated the identification of an alternate host for further EBA research. Mice with severe combined immunodeficiency (SCID) have become the preferred host for experimental propagation of aoEBA, providing a consistent bacterial load for experimental analysis (Blanchard et al., 2010).

The recent development of an effective live bacterial vaccine consisting of a quantifiable number of aoEBAinfected mouse spleen cells enables the use of vaccination as an effective tool to decrease the incidence of EBA in California. However, the difficulties and costs associated with repeatedly producing this vaccine in SCID mice stimulated interest in research to investigate alternative approaches to combat EBA. The logical long term solution for EBA abatement would be a recombinant vaccine because it would eliminate the need to propagate aoEBA in immunodeficient mice and recombinant vaccines are designed to be safer, more efficacious and less expensive than traditional vaccines (Ellis, 1999). Since recombinant vaccine development relies on a thorough understanding of the genomic makeup of the pathogen, sequencing of the aoEBA genome is the first step towards the development of an EBA recombinant vaccine.

MATERIALS AND METHODS

Tissue Collection

Four SCID mice were housed at the University of California, Davis. Studies were in compliance with UCD Institutional Animal Care Use Committee (IACUC # 17358). Animals were inoculated with 800 live cells of aoEBA and monitored by weight and body condition score for 68 days post inoculum. Animals were euthanized by CO_2 asphyxiation. Spleen tissue was collected for nucleic acid extraction.

Spleen tissue was pooled and homogenized in Hanks Balanced Salt Solution (HBSS) (Cellgro, Manassas, VA). The homogenate was passed through a 100 μ m nylon mesh (Fisher Scientific, Pittsburg, PA) and washed twice with HBSS. The cell preparation was centrifuged (1200 RPM, 10–15°C, 15 minutes) and the cells were transferred into cryomedia (80% FBS, 10% DMSO, 10% RPMI).

Nucleic Acid Extraction

The cell preparation was centrifuged (3200 RPM, 15°C, 45 minutes) and cells were resuspended in PBS. The suspension was divided into two aliquots, and the first was transferred to lysis buffer (10mM Tris pH 8.0, 0.1M EDTA pH 8.0, 0.5% SDS) containing Proteinase K. DNA was extracted by standard phenol:chloroform methods after an overnight digestion at 57°C. The second aliquot was transferred into ZR-Duet DNA/

RNA mini-prep digestion buffer (Zymo Research Corporation, Irvine, CA), and RNA was immediately extracted according to the manufacturer's protocol.

Library Preparation and Sequencing

A genomic library was prepared using the NEBNext DNALibrary Prep Kit (New England BioLabs, Ipswich, MA). The library was sequenced on the Illumina HiSeq analyzer (Illumina, San Diego, CA) and produced 243 million 100bp paired end reads (PE100). A mate-pair genomic library was prepared using Nextera Mate-Pair Protocol (Illumina, San Diego, CA) and sequenced on the Illumina HiSeq analyzer to produce 23 million PE100 reads with an average insert size of 2675 bp. The RNA was treated using RiboMinus Invitrogen Eukaryote kit for RNA (Life Technologies, Carlsbad, CA). The RNA-seq library was prepared using the NEBNext Ultra RNA Library prep kit for Illumina HiSeq analyzer (New England BioLabs, Ipswich, MA) and produced 56 million PE100 reads.

De Novo Genome Assembly

Genomic sequences from the paired end library (average insert length 230 bp) were aligned stringently to the murine genome (mm10, 11/2011) with Bowtie2 (Langmead and Salzberg, 2012) and unaligned reads were collected. The alignment to the murine genome was performed at high stringency to not remove highly conserved bacterial sequences for downstream bioinformatics analysis. Remaining reads were assembled utilizing a de Bruijn graph genome assembler, Velvet, with a hash length of 31 (Zerbino and Birney, 2008). The k-mer coverage for the mouse and bacterial genome assemblies were plotted on a histogram to determine frequency of coverage (Figure 1). The assembly was optimized utilizing only k-mer coverages consistent with the bacterial genome assembly, thus further decreasing



Figure 1. Frequency of coverage for Velvet assembly with hash length 31

¹The first peak in the bimodal distribution represents the murine contigs which have lower coverage due to the larger genome size.

²The smaller peak represents the frequency of assembled contigs originating from aoEBA. These contigs were collected for further assembly with the average coverage parameter set to 50.

murine read contamination. Eleven contiguous sequences of assembled reads (contigs) with similar coverage were collected from Velvet and nucleotide comparisons were made to the NCBI genome database to ensure contigs were not derived from the mouse genome.

Mate-pair reads were aligned to the Velvet contigs using Bowtie2, and the resulting sequence alignment map (SAM) output was used for manual contig scaffolding. To determine scaffold size and validate contig orientation, the stand-alone scaffolder SSpace was used (Boetzer et al., 2011). This program is an overlap-consensus scaffolder that utilizes paired-end and mate-pair read libraries to connect adjacent contigs inserting N's to fill the gaps. The mate pair library was determined in the SSpace output to have an average 2675 bp insert size and was utilized to scaffold contigs.

The scaffold gaps from the assembled contig were decreased by the stand-alone gap filling program GapFiller (Nadalin et al., 2012). This program utilizes the combination of the mate-pair libraries and the paired-end libraries to iteratively add base-pairs to the contigs to decrease the size of the scaffold.

All contiguous sequences were compared using Blast2Seq to visualize the consistency of assemblies at each stage using different parameters.

Annotation

The assembled genome for aoEBA was annotated using the Rapid Annotation using Subsystem Technology (RAST) (Aziz et al., 2008). The results of the annotation were manually curated to identify bacterial genes and transcripts utilizing RNA-Seq data.

RESULTS AND DISCUSSION

The de novo assembly of the aoEBA genome, a novel deltaproteobacterium, was complicated by the eukaryotic DNA contamination from the immunodeficient SCID mice in which the bacteria were grown. Although the single cell suspension was enriched with bacterial cells, the bacterium resides intracellularly and thus cannot be purified. We hypothesized that deep coverage next-generation sequencing (NGS) combined with bioinformatics analysis would overcome the eukaryotic DNA contamination and yield a complete bacterial genome.

Sequencing of the genomic library yielded a total of 243,271,075 paired-end reads from both mouse and bacterial DNA. To increase the concentration of bacterial reads within our pool of total reads, the total reads were aligned to the mouse genome (mm10, 11/2011) and the unmapped reads were collected for further analysis. A total of 98.4% of the reads mapped to the murine genome leaving 4,030,414 unmapped paired end reads. Mapping with high stringency did not remove all mouse reads; therefore, the new pool of reads consisted both murine and more concentrated bacterial reads. Despite efforts to enrich for bacterial DNA which included a variety of DNA extraction methods and cell filtering, the single cell suspension and general DNA extraction that yielded the highest concentration of bacterial

reads achieved only 1.6% of the target bacterial sequences in the total number of reads.

Sequences that did not map to the mouse genome were then used as input for the Velvet assembler. Velvet is a de Bruijn graph assembler which extracts a set of k-mers from all the reads where each node represents k-mers, and the edges represent k-1 overlapping k-mers. The Eulerian path visits the edge of each node in the graph exactly one time when the path is complete (Zerbino and Birney, 2008). The k-mer length is a parameter with a tradeoff between accuracy (large k) and coverage (small k). Velvet recommends establishing a k-mer length such that the k-mer coverage is at 20X. However, the optimal assembly, largest N_{50} , for the dataset was a k-mer length of 31 and k-mer coverage equal to approximately 50 (Figure 1). The N₅₀ value is the most commonly used statistic for assessing the contiguity of a genome assembly, where the contigs in an assembly are sorted by size and added until the total is greater than or equal to 50% of the total assembly size. The length of the contig that tips the total to over 50% is known as the N₅₀. The final Velvet assembly parameters consisted of an expected coverage of 50, minimum coverage of 25, maximum coverage of 70 and an insert length of 230. This assembly yielded 11 contigs with an N_{50} of 400,733 bp, max contig length of 683,082 bp, and total assembly of 1,815,859 bp (Table 1). The resulting contigs were compared to the NCBI nucleotide database using nblast and 10 of 11 contigs were similar to organisms of the class deltaproteobacteria. The one contig was most similar to mouse and was removed from further analysis.

The inability of Velvet to scaffold the contigs, even with the addition of mate-pair sequence information led to manual curation of the contig scaffolding. Velvet utilizes a de Bruijn approach to scaffolding and the limited coverage of the matepair data was hypothesized to inhibit Velvet from scaffolding the contigs. However, the contigs were scaffolded manually by aligning all mate-pair reads to the assembled Velvet contigs using Bowtie2 and parsing the sequence alignment map (SAM) file to pull out the 1725 mate-pair reads that aligned to two separate contigs. The SAM file provided read mapping information with the location and the contig to which the read mapped. Therefore, mate-pair mapping events, where one mate mapped to one contig and the other mate mapped to another contig, allow for the determination of contig orientation and position.

 Table 1. Assembly statistics for contiguous sequences at each stage of genome assembly

	Assembly Statistics ¹					
Method	Contigs	Total bp	N ₅₀	Ν		
Velvet	11	1,815,859	400,733	1,661		
SSpace	1	1,821,593	1,821,593	1,741		
GapFiller	1	1,821,632	1,821,632	56		

¹Assembly statistics: number of contiguous sequences (contigs), total number of base pairs assembled, contig length when the sum of contigs is greater than 50% of total assembly (N_{50}), and number of unassigned base pairs (N).

Once it was determined that all of the contigs were present to circularize the genome, the stand-alone scaffolder SSpace was used to confirm the manually curated scaffolds. SSpace utilizes an overlap based method to extend existing contigs and make scaffolds with adjacent contigs. The 63,642 (.27%) mate-pair reads that aligned to the Velvet contigs were collected and utilized alongside the paired-end reads that did not map to the murine genome for SSpace. This method was successful in extending the contigs and joining all adjacent contigs. The final assembly in SSpace was one contiguous sequence that was circularized manually resulting in a 1,821,593 bp circular genome starting at the origin of replication (TTATCCACA). The SSpace assembled genome has 1741 unknown base pairs which equates to 0.096% percent of the genome or approximately one unknown nucleotide in every 1050 base pairs. The assembled genome meets the standard for genome completion of one unknown nucleotide in each 1000 base pairs. However, the significant number of unassigned nucleotides still present in the genome led to the use of a supplementary gap filling program, GapFiller. This program utilizes a seed and extend approach for overlapping reads to the ends of segments containing unknown nucleotides and using paired-end read data to extend the existing contigs. Stringent parameters including trimming initial ends, requiring long overlaps and requiring more coverage were used to ensure accuracy of contig extensions. GapFiller initially trimmed 940 base pairs resulting in 2681 unknown nucleotides and then filled the gaps, ultimately producing a 1,821,632 base pair genome with only 56 unknown base pairs.

The resulting genome is circularized, indicating that the relative size of the genome is determined with no missing sections. When performing de novo assemblies, the relative size of the most closely related genomes are often examined to help corroborate the size of the resulting de novo assembly. *Sorangium cellulosum*, a soil bacterium that is recognized as the closest phylogenetic relative to aoEBA, has a 13 Mbp genome. Although the assembled aoEBA genome is much smaller than phylogenetically predicted, research indicates that when bacterial linages transition from free-living organisms to permanently living in a host, they rely on the host environment for many essential proteins and substantially reduce their genome size (Moran, 2002).

Lawsonia intracellularis is the only other known pathogen of the Class Deltaproteobacteria. This pathogen contains 1.72 Mbp of DNA, comprised of a 1.5 Mbp genome and 3 plasmids that are 27 kbp, 39 kbp, and 194 kbp (Sait et al., 2013). This supports the hypothesis that the 1.82 Mbp de novo assembly of aoEBA DNA represents the complete deltaproteobacterial genome, and that aoEBA has evolved significantly by reducing its genome to only those genes necessary for a pathogenic existence.

The RAST annotation pipeline was run on the assembled aoEBA genome to determine the gene profile and predict functions of the genes within the genome. RAST annotated 2250 protein coding regions, and assigned subsystems to these regions. The annotation profile of the assembled aoEBA genome (2250 coding sequences) was compared to *Sorangium cellulosum* (8576 coding sequences) to investigate evolutionary trends of the aoEBA genome. Several differences were observed in functional subsystems (metabolic pathways, protein classes, etc.) between the two species, perhaps reflecting the greater reliance of aoEBA on the host environment. Protein metabolism genes and cell wall genes are highly conserved and make up a larger percentage of the aoEBA genome compared to *S. cellulosum*. In contrast, genes contributing to iron acquisition and metabolism, and metabolism of aromatic compounds were no longer found in the aoEBA genome.

Our current results indicate that deep coverage next generation sequencing can overcome the issue of host DNA contamination from a mixed sample. With a sample containing only 1.6% percent bacterial DNA, there was enough coverage to perform a de novo assembly of a complete circular genome.

IMPLICATIONS

The development of a stable recombinant EBA vaccine would have a major impact on the California cattle industry because EBA is the leading cause of beef cattle abortion in California, responsible for the loss of an estimated 45,000 to 90,000 calves per year. Abortion rates on individual ranches can reach upwards of 50% in first calf heifers. Heifer development is a large expense for producers and late term abortion can have devastating impacts on ranch profitability. Although efforts to bring a live-bacterial vaccine into production will be an effective intermediate tool to decrease the incidence of EBA in California, the utilization of next generation sequencing techniques to study the aoEBA genome for the development of a recombinant vaccine is the logical long-term solution. This research provides the foundation to further the development of a recombinant vaccine for California's leading cause of abortion in beef cattle.

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The relationship of feed efficiency with small intestinal biology in finishing cattle¹

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ABSTRACT: The small intestine is the main site of postruminal nutrient absorption and is a major nutrient sink. Thus, physiology of the small intestine that affects nutrient absorption or use, including size, growth, blood flow, and nutrient transport, could impact whole animal feed efficiency. In a 2-yr study, small intestinal mass, growth, vascularity, and related gene expression were investigated in finishing steers classified as high or low efficiency using residual feed intake (RFI). Briefly, steers from a single contemporary group in each year were fed a finishing diet in the GrowSafe system, and individual intake was recorded. In both years, the 20% most efficient steers (n = 8/yr) and 20% least efficient steers (n = 8/yr) were selected for slaughter. Visceral organ mass data were collected, and results indicate that relative (g/kg BW) small intestinal masses tended (P = 0.09) to be decreased in high efficiency steers in yr 1 only. Histological measures of proliferation and expression of growth-related genes are currently being determined to further investigate small intestinal growth. Expression of vascular endothelial growth factor (VEGF) was greater (P = 0.002) in low efficiency compared with high efficiency steers. This suggests that VEGF, a major regulator of angiogenesis, may play a role in whole animal feed efficiency. Histological measures of vascularity are currently underway for these tissues and may provide additional information on this relationship. Jejunal expression of 7 cationic AA transporters [cationic AA transporter-1 (CAT-1), ATB^{0,+}, b^{0,+}AT and rBAT, y⁺LAT1, y⁺LAT2, and 4F2hc] and peptide transporter 1 (PepT1) was determined using real-time RT-PCR. Expression of y⁺LAT2 tended (P = 0.07) to be affected by the interaction. These data suggest jejunal expression of y⁺LAT2, a component of the y⁺L basolateral transport system of the small intestine, may contribute to differences in metabolic efficiency of cattle. Additionally, jejunal expression of glucose transporters 2 and 5 (GLUT2 and GLUT5) and sodium glucose like transporter 1 (SGLT1) was determined in this study, although no differences (P = 0.18) were observed due to efficiency class. Data collected from this study thus far suggest that

small intestinal size and gene expression may play a role in whole animal feed efficiency. Further research investigating the physiological role of the small intestine in metabolic efficiency will provide a foundation for strategies to help improve feed efficiency in beef cattle.

Key words: feed efficiency, nutrient transport, small intestine

INTRODUCTION

As feed prices have increased in recent years, there has been an increased interest in feed efficiency in the beef industry. Residual feed intake (**RFI**) is a measure of feed efficiency that is genetically independent of mature body size (Herd, 2009), in contrast to more traditional measures such as feed conversion ratio. Herd and Bishop (2000) estimated the major processes contributing to variation in RFI to include: feeding patterns (2%), digestibility (10%), body composition (5%), animal metabolism and protein turnover (37%), activity (10%), heat increment of fermentation (9%), and various other factors (27%). Despite this, the possible physiological mechanisms contributing to differences in individual feed efficiency are largely unknown.

Many of the factors estimated by Herd and Bishop (2000) are influenced by small intestinal function. The small intestine is the main site of post-ruminal nutrient absorption and has a rapid turnover rate, resulting in high nutrient and energy use. Previous data associating small intestinal mass with feed efficiency is conflicting. Basarab et al. (2003) observed that low and moderate RFI steers had decreased combined small and large intestinal mass compared with high RFI steers. Conversely, Mader et al. (2009) found that there was no relationship between RFI and total visceral, gastrointestinal, or individual visceral organ weight, even though G:F was negatively correlated with total visceral weight and positively correlated with gastrointestinal weight. Previous work in our laboratory has demonstrated that more efficient cattle in the finishing phase may have less small intestinal mass, but more dense intestinal mucosa (Meyer et

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al., 2012b).

Blood flow is crucial for nutrient transport from the small intestine to other tissues; thus vascularity of the small intestine may affect efficiency of nutrient absorption and impact feed efficiency in the whole animal. In addition, total vascularity of the small intestine has been shown to be positively correlated with feed intake in finishing steers (Cunningham et al. 2013).

Angiogenesis is the process of new blood vessel formation from pre-existing blood vessels. Vascular endothelial growth factor (VEGF) is the main growth factor that regulates angiogenesis and stimulates endothelial cell survival, proliferation, and migration in conjunction with its receptors (KDR and FLT; Klagsbrun and D'Amore, 1996). Nitric oxide (NO) induces vasodilation, which increases blood flow. Endothelial nitric oxide synthase 3 (NOS3) produces NO, and soluble guanylate cyclase (GUCY1B3) is a receptor for NO that contributes to its actions (Martin et al. 2001). Not only does NO act as a vasodilator, but it also stimulates VEGF production, acting to promote angiogenesis (Roy et al. 2006). In previous studies, small intestinal expression of these angiogenic factors has been affected by diet and intake in ruminants (Neville et al. 2010; Meyer et al. 2012a, c).

Vascularity of the small intestine is vital to transport of absorbed nutrients, but nutrients must first be absorbed by the enterocyte. Lysine, arginine, and histidine are cationic amino acids and are also 3 of the 4 most limiting AA for ruminants (Titgemeyer and Loest, 2001). There are 4 transport systems associated with 7 proteins [cationic AA transporter-1 (CAT-1, system y^+); ATB^{0,+} (system $B^{0,+}$); $b^{0,+}AT$ and **rBAT** (system $b^{0,+}$); and **y**⁺**LAT1**, **y**⁺**LAT2**, and **4F2hc** (system y⁺L)] that transport cationic AA across the apical or basolateral membrane of the enterocyte. Liao et al. (2008; 2009) reported differential expression in the 3 regions of the bovine small intestine as well as at various production stages in beef cattle. Peptide transporter 1 (PepT1) expression has also been observed in bovine small intestinal tissue, and is responsible for the transport of small peptides across the basolateral membrane (Chen, 1999; Krehbiel and Matthews, 2003). Transport of monosaccharides in the small intestine is facilitated by sodium-dependent glucose transporter 1 (SGLT1), glucose transporter 2 (GLUT2), and glucose transporter 5 (GLUT5).

Expression of cationic AA transport proteins was affected by luminal supply of glucose and AA (Liao et al., 2008, 2009). Expression of PepT1 was affected by metabolic state, where an increase in expression was observed during a nutritional challenge in rat intestinal epithelia (Thamotharan et al., 1999; Ogihara et al., 1999; Ihara et al., 2000). Moreover, Liao et al. (2010) reported that expression of the glucose transporters responds to luminal supply of glucose and is at the highest levels in the jejunum. These data suggest that nutrient availability and intake affect expression of these nutrient transporters.

Feed intake is a critical component of feed efficiency measures. Feed intake is greater for high RFI animals compared with low RFI animals across similar gains (Nkrumah et al. 2004; Kolath et al. 2006; Castro Bulle et al. 2007). Based on potential factors influencing RFI and the effect of intake and nutrient availability on vascularity and nutrient transporters, we hypothesized that small intestinal function and nutrient use may influence individual differences in feed efficiency in finishing cattle. Our objective was to investigate measures of small intestinal size, growth, vascularity, and nutrient transport in steers classified as high and low efficiency based on RFI rankings.

MATERIALS AND METHODS

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

Animal Management and Diets. Hereford-Angus crossbred steers (yr 1, n = 59, 461 \pm 4.5 kg initial BW, average age = $379 \text{ d} \pm 1.5$; yr 2, n = 75, $412 \pm 3.8 \text{ kg}$ initial BW, average age = $370 \text{ d} \pm 1.1$) from a single contemporary group in each year (birth through slaughter) were used in this study. Steers were weaned at approximately 200 d of age and grazed hay meadows until being transported to the University of Wyoming (UW) Sustainable Agriculture Research and Extension Center (SAREC) in Lingle, WY at approximately 22 d (yr 1) or 43 d post-weaning (yr 2). Upon arrival at SAREC, steers were fed a grower ration in the drylot pens. Steers were then gradually transitioned (5 rations yr 1; 4 rations yr 2) to a finishing diet consisting of 84.7% corn, 5.1% hay, 6.8% haylage, and 3.4% supplement (yr 1; DM basis) or 62.5% corn, 5.8% hay, 23.7% haylage, 4.3% straw, and 3.7% supplement (yr 2; DM basis). Monensin (Rumensin, Elanco Animal Health, Indianapolis, IN) was included in the diet to deliver 350 mg·hd⁻¹·d⁻¹ in each year.

Individual feed intake of the finishing diet (yr 1, 11.4% CP, 2.0 Mcal NE_m/kg, 1.35 Mcal NE_g/kg; yr 2, 13.2% CP, 1.8 Mcal NE_m/kg, 1.19 Mcal NE_g/kg; DM basis) was monitored using the GrowSafe system (model 4000E, GrowSafe Systems Ltd. Airdrie, AB, Canada) at SAREC for 57 (yr 1) or 80 d (yr 2). Residual feed intake was calculated as the difference between actual feed intake and expected feed intake of each individual, where expected intake was determined by regressing ADG and metabolic midweight on actual intake (Cammack et al. 2005). At the end of the feeding period in each year, steers were ultrasounded for backfat thickness, and the 20% most efficient (low RFI = high efficiency; n = 8/yr) and 20% least efficient (high RFI = low efficiency; n = 8/yr) steers with 12th rib fat thickness \geq 1.02 cm were selected for slaughter after the end of the feed intake test.

Tissue Collection. Selected steers (n = 16/yr) were randomly allocated by efficiency group to 1 of 2 slaughter dates occurring 6 and 8 d (yr 1) or 5 and 7 d (yr 2) after the end of the feed intake test. Four steers from both high and low efficiency groups were slaughtered on each day. Feed and water were not withheld from steers before transport, and steers were transported (204 km) on the morning of slaughter. Steers were slaughtered at the UW Meat Laboratory (completed in 8 h for all steers in 1 d) using standard commercial methods, and visceral organs were removed for dissection and sampling following inspection.
The small intestine was dissected, stripped of digesta and fat, and weighed using methods of Soto-Navarro et al. (2004). During dissection of the small intestine, a 10-cm jejunal sample was removed starting at a point adjacent to 15 cm caudal from the junction of the mesenteric and gastrosplenic vein on the mesenteric vein. The jejunal sample was cut open along the mesenteric side to expose the intestinal lumen and rinsed with warm PBS to remove digesta. Mucosal tissue was scraped from the jejunum using a glass slide, wrapped in aluminum foil, and flash-frozen in dry ice. Frozen tissues were stored at -80°C for later analyses.

Gene Expression. Jejunal mucosal expression of angiogenic factor [VEGF, VEGF receptors (FLT and KDR), NOS3, and GUCY1B3], cationic AA transporter [CAT-1, $ATB^{0,+}$, $b^{0,+}AT$, rBAT, v^+LAT1 , v^+LAT2 , and 4F2hc], peptide transporter 1 (PepTI), and monosaccharide transporter (GLUT2, GLUT5, SGLT-1) mRNA was determined using quantitative real-time RT-PCR (Austin et al. 2011). Primer sequences used were previously reported by Meyer et al. (2012a) and Liao et al. (2008, 2010). Frozen tissue was placed in 1 mL of TRI reagent (Sigma Chemical Co. St. Louis, MO) and homogenized using an electronic tissue grinder (IKA Laboratories; Wilmington, NC). After phase separation/ centrifugation and precipitation in isopropanol, the RNA pellet was resuspended in 100 µL RNase free water and further purified using the RNeasy kit (Qiagen, Santa Clarita, CA). The purified RNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Denver, CO).

Two micrograms of RNA (in 15 μ L nuclease-free water) were mixed with 4 μ L reverse transcription buffer (5X) and 1 μ L of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA). The mixture was placed in a thermocycler for 5 min at 25°C, 30 min at 42°C, 5 min at 85°C, and held at 4°C. The cDNA was diluted with 100 μ L nuclease-free water and stored at -20°C until semi-quantitative real-time PCR was performed. Real-time PCR was performed by mixing 10 μ L of diluted cDNA with 12.5 μ L of SYBR green Supermix (Bio-Rad Laboratories, Inc. Hercules, CA), 500 pmol each of forward and reverse primer, and 0.5 μ L of nuclease free water in each well of a 96-well plate. Amplification was performed using the iQ5 and 40 cycles of 95°C for 30 sec and

60°C for 30 sec. Melting curve analysis was performed postamplification to ensure the quality of PCR products as noted by the presence of a single peak. Briefly, the PCR plate was heated to 95°C for 3 min and cooled to 55°C, and then the temperature was increased by 0.5°C/sec up to 95°C. Bovine glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene, and all gene expression levels were quantified and reported relative to GAPDH expression using the $2^{-\Delta \Delta CT}$ method (Livak and Schmittgen, 2001). Realtime RT-PCR was performed in duplicate for each sample/ primer set.

Statistical Analysis. Data were analyzed with PROC MIXED in SAS 9.2 (SAS Inst. Inc. Cary, NC) using RFI class (high versus low efficiency), year, and their interaction as fixed effects. Post hoc analyses comparing main effect and interactive means were conducted with the LSMEANS procedure of SAS using a Tukey's adjustment and assuming an alpha of 0.05. Significance was considered when $P \le 0.05$ and tendency for $P \le 0.10$ and > 0.05. Main effects were reported in the absence of an interaction.

RESULTS

Small intestinal mass tended (P = 0.09) to be affected by the RFI class x year interaction, where there were no differences ($P \ge 0.35$) between RFI classes in each year (Table 1). In addition, small intestinal mass relative to BW (g/kg BW) was affected (P = 0.04) by the interaction of RFI class x year. There was no difference (P = 0.92) between RFI classes in yr 2, but in yr 1 low efficiency steers tended (P =0.10) to have greater relative small intestinal mass compared with the high efficiency steers.

There was a main effect of RFI class on jejunal expression of *VEGF*, where mRNA was greater (P = 0.002) in low efficiency than high efficiency steers (Table 2). Year affected (P = 0.006) *NOS3* expression, but *KDR*, *FLT1*, *NOS3* and *GUCY1B3* were unaffected ($P \ge 0.14$) by RFI class, year, or their interaction. Expression of y⁺LAT2 tended to be affected (P = 0.07) by the RFI class x year interaction (Table 3). Low efficiency steers had greater (P = 0.05) expression of y⁺LAT2 than high efficiency steers in yr 2, whereas there was no difference (P = 0.61) in yr 1. Expression of y⁺LAT1

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	RFI	class		Y	ear		P-value ²		
	Low	High							RFI x
Item	Efficiency	Efficiency	SEM ³	1	2	SEM ³	RFI	Year	Year
Small intestine, g	5,084	4,966	145	4,365	5,686	145	0.56	< 0.01	0.09
Year 1	4,601 ^b	4,129 ^{bc}	211						
Year 2	5,568ª	5,803ª	211						
Small intestine, g/kg BW	9.59	9.25	0.20	7.88	10.95	0.20	0.24	< 0.01	0.04
Year 1	8.35 ^b	7.42 ^b	0.29						
Year 2	10.83 ^a	11.08 ^a	0.29						

Table 1. Effect of residual feed intake (RFI) classification on small intestinal mass at market weight

¹ Low efficiency = 20% highest RFI steers (n = 8/yr); High efficiency = 20% lowest RFI steers (n = 8/yr).

² Means were considered significantly different where $P \le 0.05$ and tendencies for P < 0.10

³ SEM for RFI class (n = 16/efficiency class), year (n = 16/yr), or RFI class x year (n = 8/yr for each efficiency

class).

^{a-c} Interactive means (RFI x Year) differ (P ≤ 0.05).

Table 2. Effect of residual feed intake (RFI) classification on jejunal angiogenic factor mRNA

	RFI	class	Year			P-value ²			
Gene of	Low	High							RFI x
interest ³	Efficiency	Efficiency	SEM ⁴	1	2	SEM^4	RFI	Year	Year
VEGF	1.65	1.28	0.07	1.41	1.53	0.08	< 0.01	0.24	0.14
FLTI	2.10	1.81	0.18	2.17	1.74	0.19	0.28	0.11	0.85
KDR	2.43	2.13	0.19	2.27	2.28	0.20	0.29	0.97	0.86
NOS3	2.35	2.43	0.22	1.93	2.85	0.23	0.79	0.01	0.27
GUCY1B3	2.31	2.19	0.29	2.20	2.30	0.30	0.78	0.79	0.19

Low efficiency = 20% highest RFI steers (n = 8/yr); High efficiency = 20% lowest RFI steers (n = 8/yr).

² Means were considered significantly different where $P \le 0.05$ and tendencies for P < 0.10.

³VEGF = vascular endothelial growth factor, FLT1 = VEGF receptor-1, KDR = VEGF receptor-2, NOS3 = endothelial nitric oxide synthase 3, and GUCY1B3 = soluble guanylate cyclase.

SEM for RFI class (n = 16/efficiency class), year (n = 16/yr), RFI class x year (n = 8/yr for each efficiency class). ^{a-c} Interactive means (RFI x Year) differ ($P \le 0.05$).

Table 3. Effect of residual feed intake (RFI) classification on jejunal cationic AA transporter mRNA

	RFI	class		Year			P-value ²			
Gene of	Low	High							RFI x	
interest3	Efficiency	Efficiency	SEM ⁴	1	2	SEM ⁴	RFI	Year	Year	
CAT-1	2.03	2.09	0.23	2.10	2.01	0.24	0.86	0.79	0.65	
$ATB^{0,+}$	3.09	3.08	0.36	3.35	2.81	0.38	0.99	0.30	0.89	
$b^{0,+}AT$	88.2	78.8	27.0	111.0	56.2	27.9	0.81	0.17	0.57	
rBAT	2.44	2.68	0.40	2.38	2.73	0.41	0.67	0.54	0.19	
$y^{+}LATI$	4.58	3.44	0.58	5.16	2.87	0.59	0.17	0.01	0.53	
y^+LAT2	2.33	2.02	0.19	1.81	2.54	0.20	0.25	0.01	0.07	
Year 1	1.71 ^b	1.90 ^b	0.28							
Year 2	2.96 ^a	2.13 ^b	0.28							
4F2hc	2.45	1.94	0.27	1.88	2.50	0.28	0.20	0.12	0.96	
I am affection	200/ h!	sheet DEL stee	m (n - 0)	A LEAD OF	$a_{1}a_{2}a_{3}a_{4}a_{5}a_{7}a_{7}a_{7}a_{7}a_{7}a_{7}a_{7}a_{7$	M/ Louis A Di	CI attaces (a)	- 0 ()		

Low efficiency = 20% highest RFI steers (n = 8/yr); High efficiency = 20% lowest RFI steers (n = 8/yr). ² Means were considered significantly different where $P \le 0.05$ and tendencies for P < 0.10.

³Cationic AA transporter-1 (CAT-1, system y⁺); ATB^{0,+} (system B^{0,+}); b^{0,+}AT and rBAT (system b^{0,+}); and y⁺LAT1, y*LAT2, and 4F2hc (system y*L).

SEM for RFI class (n = 16/efficiency class), year (n = 16/yr), RFI class x year (n = 8/yr for each efficiency class).

^{a-c} Interactive means (RFI x Year) differ ($P \le 0.05$).

was affected by yr, where relative expression was greater (P = 0.01) in yr 1 than yr 2. Expression of SGLT1 was affected (P = 0.02) by the RFI class x year interaction, although there were no differences ($P \ge 0.12$) within each year (Table 4). Jejunal expression of GLUT2, GLUT5, and PepT1 were not affected ($P \ge 0.18$) by RFI class; however, expression of each was greater ($P \le 0.03$) in yr 2 than yr 1.

DISCUSSION

In the current study, low efficiency steers had greater small intestinal relative mass (g/kg BW) compared with high efficiency steers in yr 1, but not yr 2. These conflicting results are similar to previous reports in which small intestinal mass has been either similar (Mader et al., 2009) or greater for low efficiency steers (Basarab et al., 2003). More recently, Meyer et al. (2012b) reported that RFI was positively correlated ($P \leq$ (0.08) with small intestinal mass (r = (0.33)) and mass relative to BW (r = 0.45). This could indicate that the high efficiency animals expend less energy to maintain function of the small intestine due to decreased mass compared with the low efficiency animals. Ferrell (1988) estimates that the portal drained viscera account for 20 to 25% of whole animal energy use. Thus, decreased small intestinal mass could result in more available energy for the whole animal maintenance and growth.

In this study low efficiency steers had increased jejunal expression of VEGF. It has been reported that RFI and G:F were not correlated with histological measures of vascularity and jejunal expression of the VEGF and NO systems in a previous cattle study (Meyer et al. 2012a; Cunningham et al. 2013). Despite this, total small intestinal vascularity, as well as jejunal KDR and NOS3 expression, was positively correlated with intake in the same study (Meyer et al. 2012a; Cunningham et al. 2013). Conversely, jejunal expression of FLT1 tended to be greater in more efficient lambs in another study (Clarkson et al. 2013). Taken together, these data suggest that small intestinal expression of the VEGF and NO systems have a relationship with feed intake. This could indicate a possible relationship in angiogenesis with feed efficiency in terms of transport and uptake of nutrients.

To our knowledge, expression of the nutrient transporters investigated in this study have not been reported in relation to individual differences in feed efficiency of ruminants. Nutrient transporter synthesis and maintenance is energy demanding, which could explain the increase in v⁺LAT2 expression in the low efficiency steers compared with the high efficiency steers (Ferraris and Diamond, 1989). The decreased expression and energy use for transport in high efficiency steers may result in more available energy for maintenance and growth compared

Table 4. Effect of residual feed intake (RFI) classification on jejunal nutrient transporter mRNA

	RFI (class		Year			P-value ²			
Gene of	Low	High							RFI x	
interest ³	Efficiency	Efficiency	SEM ⁴	1	2	SEM ⁴	RFI	Year	Year	
GLUT2	8.17	6.68	1.19	3.12	11.73	1.23	0.38	< 0.01	0.24	
GLUT5	1.96	1.64	0.17	1.54	2.06	0.17	0.18	0.03	0.14	
SGLT1	6.14	5.01	0.81	3.73	7.42	0.84	0.33	< 0.01	0.02	
Year 1	2.92 ^c	4.54 ^{bc}	1.18							
Year 2	9.37 ^a	5.48 ^{ab}	1.18							
PepTI	6.72	5.47	0.85	4.33	7.86	0.87	0.31	< 0.01	0.85	

¹ Low efficiency = 20% highest RFI steers (n = 8/yr); High efficiency = 20% lowest RFI steers (n = 8/yr).

² Means were considered significantly different where $P \le 0.05$ and tendencies for P < 0.10.

³ GLUT2 = Glucose transporter 2, GLUT5 = Glucose transporter 5, SGLT1 = Sodium glucose transporter 1, PEPT1 = Peptide transporter 1.

⁴ SEM for RFI class (n = 16/efficiency class), year (n = 16/yr), RFI class x year (n = 8/yr for each efficiency class). ^{ac} Interactive means (RFI x Year) differ ($P \le 0.05$).

with their low efficiency counterparts. In addition, in our study (data not shown) and other studies, low efficiency (high RFI) steers have increased intake (Nkrumah et al. 2004; Kolath et al. 2006; Castro Bulle et al. 2007) compared with their high efficiency counterparts, which could indicate an increase in luminal nutrient supply. Liao et al. (2009) demonstrated differences in expression of the cationic AA transporters relative to luminal supply of AA and glucose.

While expression of PepT1 and the 3 monosaccharide transporters were unaffected by efficiency class within each year, this data suggests that further investigation is required to better understand the role that nutrient transport may have on feed efficiency. Due to diet composition, very little starch would have been available for further digestion and absorption in the small intestine in this study, which may have influenced our results as well. Little absorption of glucose occurs in the small intestine of ruminants due to the digestion of starch and carbohydrates in the rumen, it is reported that the greatest absorptive capacity for glucose post-ruminally occurs in the jejunum (Krehbiel et al., 1996, Harmon and McLeod, 2011). Additionally, there is potential for differences to exist in transport kinetics, binding affinity, and cell turnover that were not measured in this study that could influence individual differences in feed efficiency.

Further analyses planned for small intestinal tissues from this study include histological measures of vascularity and growth and determination of cellularity. These may provide additional insight into the role of small intestinal size and function in efficiency of feed utilization. In conclusion, data from the current study suggest that small intestinal mass and gene expression related to blood flow and nutrient transport could contribute to individual differences in feed efficiency.

IMPLICATIONS

Feed efficiency is of economic interest to beef producers. With recent record-high feed prices, decreased quantity and quality of forage due to drought, increased demand for product, and progressively limited available land, it is vital to understand physiological mechanisms that impact an overall metabolic efficiency. It is critical for cattle to have high production levels, yet financially it is also vital for producer to control inputs without negatively impacting performance. It is well known that individual animal intake differences exist even at similar production levels. However, many physiological mechanisms of this increased efficiency of nutrient utilization are unknown. If these mechanisms that influence efficiency of absorption and utilization of these nutrients are better understood, they may be used to develop management strategies to improve feed efficiency.

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Maternal nutrient restriction followed by realimentation during early to mid-gestation on uterine blood flow and conceptus development in beef cows

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INTRODUCTION

ABSTRACT: In order to determine the effect of maternal nutrient restriction followed by realimentation during early to mid-gestation on uterine blood flow (BF), maternal performance, and conceptus development in pregnant beef cows 2 experiments were conducted. Effects of maternal nutrient restriction followed by realimentation during midgestation on uterine BF of lactating, multiparous cows were evaluated during experiment 1. Nutrient restriction from d 30 until 140 of gestation did not alter total uterine BF. However, from d 140 to 198 of gestation when cows were realimented, there was enhanced ipsilateral uterine BF. In Experiment 2, effects of maternal nutrient restriction followed by realimentation during early to mid-gestation on late gestation uterine BF, maternal performance, and conceptus development was evaluated using non-lactating, multiparous cows. Slaughters were performed at d 85, 140, and 254 of gestation. During late gestation when a subset of cows were receiving similar nutrition (100% of the NRC requirements), ipsilateral uterine BF and total BF were increased in cows that were previously nutrient restricted from d 30 until d 85 of gestation and realimented until d 254 of gestation. Therefore, results from both experiments suggest that the bovine placenta may be programmed to function differently after a period of nutrient restriction. Duration of restriction or realimentation impacted maternal performance and organ weights. The dam might become more efficient in the utilization of nutrients after being realimented as gestation advances. Nutrient restriction during early pregnancy tended to increase fetal and placental size by d 85. However, when cows were restricted longer or when realimented, there were no observable differences in placental or fetal growth. The maternal system may adapt to allow for fetal catch up growth during later gestation by enhancing uteroplacental nutrient transport capacity or placental function. From these experiments we can conclude that maternal nutrient restriction during early gestation enhances conceptus growth and uterine BF later in pregnancy. Perhaps, timely management strategies might result in enhanced conceptus development. Even though more research is necessary, opportunities to intervene appear to be available during times of poor nutrition in beef cow/ calf systems.

Key words: Beef cattle, blood flow, nutrient restriction

Intrauterine growth restriction is associated with altered fetal organ development and subsequent performance of offspring (Godfrey and Barker, 2000; Wu et al., 2006). As postnatal growth is largely dependent upon fetal growth and development in utero, it is important to determine how management decisions could impact the growth trajectory of the bovine fetus. Even during the time of early embryonic development, when nutrient requirements appear trivial for conceptus growth, maternal nutrition can alter organogenesis and establishment of the placenta, which are imperative for proper prenatal growth and development (Robinson et al., 1999).

The placenta plays a major role in the regulation of fetal growth. Placental nutrient transport efficiency is directly related to utero-placenta blood flow (Reynolds and Redmer, 1995). Gases, nutrients, and metabolic end products are exchanged between maternal and fetal circulation via the placenta (Bleul et al., 2007; Reynolds and Redmer, 1995; Reynolds and Redmer, 2001). Increases in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on growth of the placenta during early pregnancy followed by dramatic development and reorganization of the uteroplacental vasculature during the last half of gestation (Meschia, 1983; Reynolds and Redmer, 1995). If an insult (i.e. maternal nutrient restriction) during early gestation impairs placental development, calf development during later pregnancy could be altered. We have shown (Camacho et al., 2014b) that the dam undergoes adaptations when nutrient restriction occurs during early to mid-gestation. It appears that the multiparous cow becomes more efficient in the utilization of nutrients after being realimented and as gestation advances providing sufficient nutrients for the developing conceptus (Camacho et al., 2014b).

We have previously reported (Camacho et al., 2014a) that nutrient restriction (60% of NRC requirements) in lactating Simmental cows from d 30 to 140 of gestation does not alter total uterine BF. However, upon realimentation, ipsilateral uterine BF is enhanced until d 198 of gestation. It is still unclear how realimentation after various lengths of restriction would impact uterine blood flow during gestation.

The bovine uteroplacenta may compensate during nutrient restriction and be programmed to function differently after the insult. Therefore, we hypothesized that nutrient restriction during early to mid-gestation in beef cows would impact placental and fetal growth development throughout gestation. Moreover, we further hypothesized that upon nutrient realimentation the placenta would become more efficient and uterine BF would surpass the BF from control animals. The specific objective was to examine the effect of maternal nutrient restriction followed by realimentation during early to mid-gestation on uterine artery hemodynamics as well as conceptus development.

MATERIALS AND METHODS

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

Animals, Diets, and Breeding

A total of 54 non-lactating, multiparous crossbred beef cows (initial BW = 620.5 ± 11.3 kg, BCS = 5.1 ± 0.1) of similar genetic background were synchronized using a Select Synch plus progesterone insert (CIDR; Pfizer Animal Health, New York, NY) and fixed-time AI (TAI). The breeding protocol has been previously published (Camacho et al., 2014b). Inseminated cows were transported to the Animal Nutrition and Physiology Center (ANPC; Fargo, ND) within 3 d postinsemination. On d 27 and 28 post-insemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). Non-pregnant cows restarted the same breeding protocol; cows were only subjected to AI twice during the experiment; if not pregnant after the second AI, cows were not utilized for the experiment. On d 30 of pregnancy, cows were randomly assigned to dietary treatments (n = 4 to 5 per pen with greater than 1 dietary treatment per pen): control (CON; 100% NRC; n = 18) and nutrient restriction (RES; 60% NRC; n = 30). On d 85, cows were slaughtered (CON, n = 6 and RES, n = 6), remained on control (CC; n = 12) and restricted (RR; n = 12) treatments, or were realimented to control (RC; n = 11). On d 140, cows were slaughtered (CC, n = 6; RR, n = 6; RC, n = 5), remained on control (CCC, n =6; RCC, n = 5), or were realimented to control (RRC, n = 6). On d 254, all remaining cows were slaughtered (CCC, n = 6; RCC, n = 5; RRC, n = 6). An animal from the RC group was removed from the study due to early embryonic loss and a second cow was removed from the RCC group due to a twin pregnancy.

The control diet consisted of grass hay fed to meet 100% NE recommendations for maintenance and fetal growth (NRC, 2000) and to meet or exceed MP, mineral, and vitamin recommendations. Nutrient restricted cows received 60% of the same control hay diet. Cows were individually fed once daily in a Calan gate system at 1000 h and had free access to water. The mineral and vitamin supplement (Trouw Dairy VTM with Optimins; Trouw Nutrition International, Highland, IL; 10% Ca, 5% Mg, 5% K, 2.7% Mn, 2.7% Zn,

1,565,610 IU/kg vitamin A, 158,371 IU/kg vitamin D 3 and 2,715 IU/kg vitamin E) was top-dressed 3 times per week at a rate of 0.18% of hay DMI to meet or exceed mineral and vitamin requirements relative to dietary NE intake (NRC 2000). Cows were weighed weekly at approximately 0800 h throughout the experiment. Initial and final BW were taken on 2 consecutive days. Dietary intake was adjusted relative to BW weekly and to NE requirements for the specific period of gestation (average requirements for periods from d 30 to 85, d 86 to 140, 141 to 197, and d 198 to 254).

Ultrasonography Evaluation

A subset of cows (i.e. all the CCC, RCC, and RRC cows) were examined via ultrasonography within 2 d of every reported sampling time. Uterine artery hemodynamics, ipsilateral and contralateral to the fetus, were obtained via color Doppler ultrasonography (model SSD-3500; Aloka America, Wallingford, CT) fitted with a 7.5 MHz finger transducer (Aloka UST-995) on d 210, 225, and 240 of gestation. Briefly, the probe was inserted through the rectum and the aorta was located. In B mode using the finger probe, the origin of the external iliac, ipsilateral to the gravid uterine horn, was located and the transducer was moved caudally to locate the internal iliac artery. The maternal umbilical artery begins as a major branch of the internal iliac, and gives rise to the uterine artery (Bollwein et al., 2000). After the uterine artery was identified as a movable and pulsating artery, a longitudinal section was visualized by manually turning the transducer of the probe. The probe was aligned to the uterine artery at an average angle of insonation of 79 ± 0.2 degrees and uterine artery hemodynamic measurements were collected.

For each uterine artery BF, 3 similar cardiac cycle waveforms from 3 separate ultrasonography evaluations from each side (ipsilateral and contralateral uterine artery) were obtained with spectral Doppler and averaged per cow within a gestational day (i.e. 9 measurements per side per artery per sampling day). Pulsatility index (**PI**), resistance index (**RI**), and uterine and umbilical artery blood flow (**BF**) were calculated by pre-programmed Doppler software where PI = (peak systolic velocity - end diastolic velocity)/ mean velocity; RI = (peak systolic velocity - end diastolic velocity)/ peak systolic velocity; and BF (mL/min) = mean velocity (cm/s) × ($\pi/4$) × cross-sectional diameter (cm²) × 60 s. Total BF was calculated as the sum of ipsilateral and contralateral uterine artery BF.

Slaughters and Tissue Collection

A subset of pregnant cows from each breeding group was randomly selected for slaughter at d 85, 140, and 254 (\pm 2 d SD). Cows were transported from ANPC to the NDSU Meat Laboratory approximately 30 min prior to slaughter. No more than 2 cows were slaughtered per day due to time constraints of sample collection (slaughters ranged from November 2011 until April 2012). On the day of slaughter, cows were stunned with a captive-bolt gun and exsanguinated. The gravid uterus was immediately collected and weighed. The fetus was immediately removed from the placenta at the umbilicus and weighed. Chorioallantoic and amniotic fluids were combined and volume was recorded. After each individual placentome was weighed, placentome dimensions [length (l), width (w), depth (d)] were measured using digital calipers and were separated manually into cotyledonary and caruncular portions. The mass of total cotyledonary tissue and total caruncular tissue was recorded. Average placentome density (g/cm³) was calculated as placentome weight divided by placentome volume ($l \times w \times d$). After all placentomes and fetal membranes were removed, the uterus was reweighed to obtain an empty uterine weight.

Statistical Analysis

Uterine BF was analyzed using the repeated measures analysis of the MIXED procedure of SAS (SAS software version 9.2, SAS Inst., Cary, NC). The model included treatment, day, and treatment by day interaction. Breeding group was used as a block and appropriate covariance structures were selected. Within each slaughter day, conceptus measurements were analyzed using the MIXED procedure of SAS (SAS software version 9.2, SAS Inst., Cary, NC). The model statement included treatment and fetal sex. When a significant treatment effect was detected ($P \le 0.05$), treatment differences were separated using the PDIFF option of the LSMEANS statement.

RESULTS

Late Gestation Uterine BF. There was no treatment \times day interaction (P = 0.36; Fig. 1A) for ipsilateral uterine artery BF in cows that were slaughter at d 254. However, there was a treatment (P = 0.02) and a day effect (P = 0.01). Cows from the RCC treatment had greater ipsilateral uterine artery BF compared to CCC and RRC cows. In addition, ipsilateral uterine BF increased in all treatment groups during late gestation. For ipsilateral uterine artery CSA, there was no treatment \times day interaction (P = 0.96; Fig. 1B) or main effect of treatment (P = 0.70) but there was a day effect (P < 0.01). Ipsilateral uterine artery CSA increased during late gestation regardless of dietary treatment. For ipsilateral uterine artery PI, there was no treatment \times day interaction (P = 0.79; data not shown) or main effect of day (P = 0.22). However, there was a tendency for ipsilateral uterine artery PI to decrease (P = 0.09) in RCC and RRC cows compared to CCC cows. Similarly, ipsilateral uterine artery RI did not display a treatment \times day interaction (P = 0.60; data not shown) or main effect of day (P = 0.36). However, ipsilateral uterine artery PI decreased (P = 0.03) in RCC and RRC cows compared to CCC cows.

There was no treatment × day interaction (P = 0.92; Fig. 1C) or treatment effect (P = 0.33) for contralateral uterine artery BF. However, there was a day effect (P = 0.01) where



Figure 1. Ipsilateral uterine artery blood flow (BF; panel A), ipsilateral cross-sectional area (CSA; panel B), contralateral uterine artery blood flow (BF; panel C), contralateral cross-sectional area (CSA; panel D) during late gestation. Subset of cows were selected (CCC, n = 6; RCC, n = 5; RRC, n = 6) and uterine artery hemodynamics were obtained during late gestation.

contralateral uterine BF increased in all treatment groups during late gestation. For contralateral uterine artery CSA, there was no treatment × day interaction (P = 0.97; Fig. 1D) or main effect of day (P = 0.14) but there was a tendency (P =0.09) for a treatment effect. Contralateral uterine artery CSA tended to decrease in RCC cows compared to CCC and RRC during late gestation. For contralateral uterine artery PI, there was no treatment × day interaction (P = 0.84; Fig. data not shown) or main effect of treatment (P = 0.65) or day (P =0.20). Similarly, for contralateral uterine artery RI there was no treatment × day interaction (P = 0.98; data not shown) or main effect of treatment (P = 0.26) or day (P = 0.84).

There was no treatment × day interaction (P = 0.34; Fig. 2) for total uterine artery BF, but there was a main effect of treatment (P = 0.05) where total uterine artery BF was increased in RCC cows vs. CCC and RRC cows. In addition, there was a day effect (P < 0.01) where all cows had increased total uterine artery BF during late gestation.

Placental and Fetal Measurements at d 85. Fetal weight tended to be increased (P = 0.07) in RES cows vs. CON cows. Gravid uterine weight was not affected (P = 0.19) by treatment at d 85. However, empty uterine weight tended (P = 0.09) to be greater in RES cows compared to CON cows. Fetal membrane weight and chorioallantoic and amniotic fluid volume were similar ($P \ge 0.45$) between treatments. The number of placentomes was increased (P = 0.02) in RES compared to CON cows, and therefore the total mass of the placentomes was greater (P < 0.01) in RES compared to CON cows. Total cotyledon weight and total caruncle weight were not affected ($P \ge 0.27$) by maternal dietary treatment. Average placentome weight and average placentome volume and density were similar ($P \ge 0.51$) between treatments.

Placental and Fetal Measurements at d 140. Fetal weight was similar (P = 0.54) among treatments. Gravid and empty uterine weight were similar ($P \ge 0.86$) among treatments.



Figure 2. Total uterine artery blood flow (BF) during late gestation. Subset of cows were selected (CCC, n = 6; RCC, n = 5; RRC, n = 6) and measurements were obtained via Doppler ultrasonography.

Fetal membrane weight and chorioallantoic and amniotic fluid volume were also similar ($P \ge 0.63$) among treatments. Total number of placentomes was greater (P = 0.03) in RR cows compared to CC and RC cows; however, total weight of placentomes was not affected (P = 0.18) by maternal dietary treatment. In addition, total cotyledon weight and total caruncle weight were similar ($P \ge 0.51$) among treatments. Average placentome weight, average placentome volume and density were similar ($P \ge 0.14$) among treatments.

Placental and Fetal Measurements at d 254. Fetal weight was similar (P = 0.84) among treatments. Gravid and empty uterine weight were similar ($P \ge 0.29$) among treatments. Fetal membrane weight increased (P = 0.04) in RRC cows compared to CCC and RCC cows. Chorioallantoic and amniotic fluid volume were also similar (P = 0.61) among treatments. Total number of placentomes and total weight were not affected ($P \ge 0.11$) by maternal dietary treatment. In addition, total cotyledon weight similar (P = 0.55) among treatments; however, total caruncle weight tended to be greater in RRC cows compared to CCC and RCC cows. Average placentome weight also tended to be greater (P = 0.09) in RRC cows compared to CCC and RCC cows. However, average placentome volume and density were similar ($P \ge 0.15$) among treatments.

DISCUSSION

We hypothesized that maternal nutrient restriction followed by realimentation from early to mid-gestation in beef cows would impair placental and fetal development and late gestation BF. We observed a tendency for fetal growth to be increased at d 85 in RES cows compared to CON cows. Therefore, it appears that stunting of fetal growth was spared due to increased placental growth in restricted cows. Interestingly, there was a decrease in umbilical BF relative to fetal weight at d 85 in RES fetuses compared to CON. This decreased blood perfusion might explain the similarity in fetal weights in nutrient restricted dams realimented at d 140 and 254. However, when cows were realimented during early and mid-gestation we no longer observed treatment effects for placental or fetal weight. Previous research in beef cows, with a nutrient restriction to 50% of the requirements from d 30 to d 125 of gestation reduced caruncular and cotyledonary weight compared to control cows at the end of the restriction (Zhu et al., 2006). However, when cows were realimented caruncular weight was similar between restricted and control cows at d 250 (Zhu et al., 2007). Vonnahme et al. (2007) demonstrated that restriction from d 30 to 125 did not affect placental vascularity. Conversely, upon realimentation, placental vascularity was altered near term, indicating that the placenta compensated after restriction. In our study, cows were slaughtered at d 85 of gestation following 55 d of maternal nutrient restriction (from d 30 to d 85 gestation). We observed differences in placentome weight and number where RES cows had greater mass and greater number of placentomes than CON cows at d 85 of gestation. Therefore, 55 d of maternal nutrient restriction during early gestation appears to lead to increased placental growth and development in RES compared to CON cows. However, at d 140 of gestation the only effect observed was for number of placentomes being increased in RR cows compared to CC and RC and at d 254 of gestation there were no differences in placentome numbers due to maternal dietary treatment. Vonnahme et al. (2007) observed dramatic differences in capillary vascularity after the realimentation period (from d 125 to 250), suggesting an alteration of placental development and function by early nutrient restriction. In a companion study to the current study, Reyaz et al. (2012) showed that cotyledonary arteries from RES cows were more sensitive to bradykinin-induced vasorelaxation compared to cotyledonary arteries from CON cows. Rasby et al. (1990) manipulated diets to achieve low and moderate BCS in pregnant cows from d 195 to 259 of gestation followed by slaughter at d 260 of gestation. They observed similar fetal weight and caruncular number and weight between groups; however, uterine weight was greater and amnionic fluid volume tended to increase in moderate BCS cows compared to thin BCS while cotyledonary and fetal membranes weight were greater in thin cows compared to moderate BCS cows. Even though nutrient restriction occurred during late gestation (Rasby et al., 1990), it is important to note that the placenta compensates due to reduction in intake and body energy reserves.

Reynolds et al. (2006) summarized several studies that used sheep as a model of compromised pregnancies during late gestation (i.e. overfed and underfed dams, heat and hypoxia stress, multiple pregnancies) where most of the studies showed a decrease in umbilical BF and a decrease in fetal or placental weight or both. More specifically, nutrient restriction in sheep during early gestation (from d 28 to 78) resulted in intrauterine growth restriction in restricted vs. control fed ewes (Vonnahme et al., 2003). In human fetuses, abnormal umbilical artery BF during late gestation indicates intrauterine growth restriction, suggesting high risk for perinatal death (Kingdom et al., 1997). Currently, a paucity of research exists on umbilical hemodynamics in cattle; however, if we are able to determine abnormal umbilical wave forms during early gestation in cows we might be able to elucidate strategies to improve neonatal health. In this study we observed that d 85 fetuses from RES cows tended to be heavier compared to fetuses from CON cows suggesting an increased nutrient extraction from the nutrient restricted dams. As umbilical BF was similar between treatments, we hypothesize that nutrient uptake by the placenta in nutrient restricted animals must be enhanced. However, when cows were realimented at d 85 and 140 of gestation fetuses had similar BW and also umbilical BF was similar among treatments.

Interestingly, during late gestation when all cows were receiving similar nutrition (100% of NRC recommendations), ipsilateral uterine BF and total BF were increased in cows that were nutrient restricted for 55 d and then realimented. Our laboratory has previously reported (Camacho et al., 2014a) that nutrient restriction from d 30 to 140 followed by

Table 1. Nutrient analysis of grass hay

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

realimention in beef cows increases ipsilateral uterine artery BF, however, total uterine BF was not affected by nutrient restriction. The fact that we observed changes in total uterine BF during late gestation but not during mid-gestation due to nutrient restriction followed by realimentation might be due to the exponential increase of BF that occurs during late gestation. Late gestation is characterized by a well-known rapid increase in fetal growth and development and subsequent increase in nutrient demands of the fetus. Therefore, nutrient uptake by the gravid uterus and fetal energy deposition are the greatest during late gestation (Reynolds et al., 1986). In order to meet the nutrient demands, vascular smooth muscle tone of the uterine artery decreases causing an increase in diameter of the artery and subsequent increase in BF (Rosenfeld, 1984; Ford, 1995).

In summary, nutrient restriction during early restriction tended to increase fetal growth and placental size. However, when cows were restricted longer and/or realimented during early and mid-gestation we no longer observe treatment effects for placental or fetal growth. This suggests that after a longer period of restriction, fetal growth catches up with the early restricted cows and this might be due to nutrient transport capacity or placental function. It is known that pregnancy success and offspring health are dependent on nutrient intake and conceptus growth and development. A key player for proper conceptus development is the placenta as it plays an important role in providing physiological exchange between the maternal and fetal systems (Reynolds and Redmer, 1995). During placentation, angiogenesis and vascularization at the fetal-maternal interface is extensive and, subsequently, a rapid increase in uterine and umbilical BF results (Reynolds and Redmer, 1995). In order to support the growth of the developing fetus during late gestation, even though the placenta is not growing as much as early gestation, placental function increases dramatically after mid-gestation (Reynolds et al., 1986).

IMPLICATIONS

It appears that cattle may be more tolerant of inadequate nutrition during early to mid-pregnancy compared to sheep. In addition, the bovine placenta may be programmed to function differently after a period of nutrient restriction by partitioning nutrients/resources more towards the placental and fetal growth. Therefore, opportunities may be available to intervene during times of poor nutrition.

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Incorporation of sexed semen into reproductive management of cow-calf operations

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INTRODUCTION

ABSTRACT: The objective of this experiment was to compare reproductive performance and weaning outcomes of beef cows inseminated with sexed or conventional semen. Over 2 consecutive yr, lactating Angus × Hereford cows were assigned to an estrus synchronization + AI protocol. At the time of AI, cows were ranked by parity and assigned to be inseminated with conventional non-sorted semen (CONV; n = 454) or with semen sorted for male sperm (SEXED; n =439). Beginning 18 d after AI, cows from both treatments were grouped and exposed to mature bulls for 50 d (1:25 bull to cow ratio). Cow pregnancy status to AI was verified by detecting a fetus via transrectal ultrasonography 40 d after AI. Calf birth date, sex, and birth BW were recorded during the subsequent calving season. Cows that were diagnosed as pregnant during the transrectal ultrasonography exam and gave birth during the initial 2 wk of the calving season were considered pregnant to AI. Pregnancy rates to AI and final pregnancy rates (AI + bull breeding) were reduced ($P \le 0.05$) in SEXED compared with CONV cows. The proportion of male calves born to AI or AI + bull breeding was greater (P <0.01) in SEXED compared with CONV cows. No treatment effect was detected (P = 0.34) for weaning rate, whereas SEXED cows had a greater (P < 0.01) proportion of steers in the weaned calf crop compared with CONV cows. Steers and heifers from SEXED cows were younger (P < 0.01), whereas only SEXED heifers were lighter (P = 0.05) at weaning compared with cohorts from CONV cows. Across genders, calves from SEXED cows had reduced ($P \le 0.01$) weaning age and BW compared with calves from CONV cows. Cows assigned to SEXED had greater (P=0.05) kg of steer weaned/ cow exposed to breeding, but reduced kg of heifer weaned/ cow exposed to breeding (P < 0.01) compared with CONV cows. Across genders, SEXED cows tended (P = 0.09) to have reduced kg of calf weaned/cow exposed to breeding compared with CONV cows. In summary, inseminating beef cows with sexed semen reduced pregnancy rates, but increased the proportion of steers weaned and kg of steers weaned/cow exposed to breeding. However, overall kg of calf weaned/cow exposed to breeding was not improved by the use of sexed semen, particularly because of its negative impacts on weaning age and BW of the heifer progeny.

Keywords: Artificial insemination, beef cattle, reproduction, sexed semen, weaning parameters.

The major objective of cow-calf systems is to produce 1 calf per cow annually. Therefore, profitability of cowcalf operations is primarily determined by reproductive performannce of the cowherd, which defines the number of calves born and weaned annually (Wiltbank et al., 1961). Economic returns in cow-calf systems can also be increased by adding quality and value to the weaned calf crop, which can be accomplished via breeding strategies such as inseminating the cowherd with sexed semen. More specifically, steers have greater weaning and yearling BW compared with contemporary heifers (Koch and Clark, 1955; Koger and Knox, 1945). In addition, average value/kg of live BW was 10 % greater for feeder steers compared with feeder heifers during the last 5 yr in the U.S. (USDA-Agricultural Marketing Service, 2013). Therefore, we hypothesized that inseminating beef cows with semen sorted for male sperm benefits economic returns in cow-calf operations by increasing the proportion of steers available for marketing after weaning.

Nevertheless, early research demonstrated that sexed semen yield reduced pregnancy rates when compared to conventional semen (Seidel, 2007), which may prevent optimal reproductive performance of the cowherd and annul the potential benefits on calf crop value. However, with recent advances in semen sorting and freezing, some research has suggested that pregnancy rates to sexed semen are improving and reaching comparable results to conventional semen (Hall et al., 2010), although additional studies with larger groups of beef cattle are warranted to validate this outcome. Further, no research has assessed the impacts of inseminating beef cows with sexed semen on calf crop performance and overall weaning returns in cow-calf systems. Therefore, the objective of this experiment was to compare reproductive performance and weaning outcomes of lactating beef cows inseminated with sexed or conventional semen.

MATERIALS AND METHODS

This experiment was conducted over 2 consecutive yr (2011 and 2012) at the Oregon State University (OSU) – Eastern Oregon Agricultural Research Center (EOARC; Burns station and Union station). In 2011, a total of 441 lactating Angus × Hereford cows were enrolled in the experiment (Burns, n = 209 multiparous and 34 primiparous; Union, n = 149 multiparous and 49 primiparous). In 2012, a total of 452 lactating Angus × Hereford cows were enrolled (Burns, n = 196 multiparous and 49 primiparous; Union, n = 160 multiparous and 47 primiparous). Moreover, 345 cows were used in both yr (Burns, n = 194, Union, n = 151), 96 cows were used only in 2011 (Burns, n = 49, Union, n = 47), and 107 cows were used only in 2012 (Burns, n = 51, Union, n = 56). All cows and calves utilized herein were managed as described by Cooke et al. (2012), and 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. All cows were assigned to an estrus synchronization + AI protocol. Cows received 100 µg of GnRH (Factrel; Zoetis, Florham Park, NJ, USA) plus a controlled internal device release (CIDR) containing 1.38 g of progesterone (Zoetis), followed in 7 d with 25 mg of prostaglandin F2a (Lutalyse; Zoetis) and CIDR removal, followed in 60 h by a second 100 µg injection of GnRH and AI. At the time of AI, cows were ranked by parity and assigned to be inseminated with conventional non-sorted semen (CONV; n = 360 multiparous and 94 primiparous; Genex Cooperative, Inc., Shawano, WI, USA) or with semen sorted for male sperm (SEXED; n = 354 multiparous and 85 primiparous; GenChoice 90TM, Genex Cooperative, Inc.). At the Union station, cows that displayed estrus beginning after the prostaglandin F2 α injection and until 24 h before the second GnRH injection were inseminated 12 h after estrus detection (n = 56 for CONV and 51 for SEXED), whereas all other cows were timed-AI at the time of the second GnRH injection (n = 151 for CONV and 147 for SEXED). The CONV semen contained approximately 20 million non-sorted sperm cells per straw, whereas SEXED contained approximately 2.1 million sperm cells per straw with 90 % of these sperm cells expected to be male sperm (Rath and Johnson, 2008). Within each yr and location, cows were inseminated by the same technician with CONV or SEXED originated from the same bull. The Burns station cowherd was inseminated with semen from Club King (1SM00115, Genex Cooperative, Inc.) in 2011 and Upgrade (1SM00121; Genex Cooperative, Inc.) in 2012, whereas the Union station cowherd was inseminated with semen from Chisum (1AN01170; Genex Cooperative, Inc.) during both yr. Beginning 18 d after AI, all cows from both treatments were grouped and exposed to mature Angus and Hereford bulls (age = 5.6 ± 0.4 yr) for 50 d (1:25 bull to cow ratio). All bulls utilized in this experiment were submitted to and approved by a breeding soundness evaluation (Chenoweth and Ball, 1980) before the breeding season.

Sampling. Cows were evaluated for BCS at the time of AI (Wagner et al., 1988). Blood samples were collected concurrently with AI and 7 d later for determination of plasma progesterone concentration to assess estrus synchronization rate. Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA) containing sodium heparin (158 USP units), placed on ice immediately, and centrifuged at $2,400 \times g$ for 30 min at

room temperature for plasma collection. Plasma was stored at -80° C on the same day of collection. Plasma progesterone concentration was determined as described by Munro and Stabenfeldt (1984) with modifications described by Galvão et al. (2004). The intra- and inter-assay CV were 7.8 and 6.0 %, respectively, whereas assay sensitivity was 0.05 ng/mL. Cows with plasma progesterone concentration < 1.0 ng/mL at AI, but \geq 1.0 ng/mL 7 d later were classified as responsive to the estrus synchronization protocol.

Cow pregnancy status to AI was verified by detecting a fetus via transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka) 40 d after AI. During the subsequent calving season, calf birth date, sex, and birth BW were recorded. Pregnancy loss during gestation was not accounted for in the present experiment because cow pregnancy status was not evaluated after the end of bull breeding. Hence, all cows that gave birth during the calving season were classified as becoming pregnant during the experiment. Calf paternity (AI or bull breeding) was determined according to transrectal ultrasonography and birth date. Only cows that were diagnosed as pregnant during the transrectal ultrasonography exam and gave birth during the initial 2 wk of the calving season were considered pregnant to AI. Calves that died at birth or up to weaning were accounted for as calf loss from birth to weaning. No incidences of dystocia were observed in the present experiment. Calf BW was determined again at weaning, whereas 205-day adjusted weaning BW was calculated according to BIF (2010). Calf weaning value was estimated based on US\$/kg of BW within 45.5 kg increments for feeder steers and heifers (from 114 to 386 kg of BW), according to the latest 5-yr U.S. average (2008 to 2012; USDA-Agricultural Marketing Service, 2013).

Statistical analysis. All data were analyzed with cow as the experimental unit and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. All quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), whereas binary data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). Model statements contained the effects of treatment, parity, yr, location, calf gender (for analyses containing calf parameters), and all resultant interactions. The random statements contained the effect of cow (treatment \times location \times yr \times parity), or cow(treatment \times location \times yr \times parity \times calf gender) for analyses containing calf parameters. However, analyses of cow age at AI, as well as quantitative and binary data containing 205-day weaning BW, did not contain the effects of parity in the model and random statements. Pregnancy rates to AI at the Union station were also analyzed using a model statement containing the effects of treatment, parity, yr, AI method (after estrus detection or fixed-time AI), all resultant interactions, and with a random statement containing the effect of $cow(treatment \times yr \times parity)$ × AI method). Results are reported as least square means and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if P > 0.05 and ≤ 0.10 . Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

RESULTS AND DISCUSSION

Overall reproductive results. No treatment effects were detected ($P \ge 0.51$) for cow BCS and age at AI, as well as estrus synchronization rate (Table 1). Hence, all treatment effects reported herein were independent of these parameters. Pregnancy rates to AI were reduced in SEXED compared with CONV cows (Table 2), independently if analysis contained all cows exposed to AI (P < 0.01) or only cows that were effectively synchronized to the estrus synchronization protocol (P < 0.01). Within the Union station, SEXED cows had reduced ($P \le 0.05$) pregnancy rates to AI compared with CONV cows independently if cows were inseminated 12 h after estrus detection (53.6 vs. 74.5 % for all cows, SEM = 6.7%; 57.4 vs. 76.0 % for synchronized cows, SEM = 6.7 %; for SEXED and CONV, respectively) or without estrus detection at fixed-time AI (42.6 vs. 56.3 % for all cows, SEM = 4.0%; 48.9 vs. 68.5 % for synchronized cows, SEM = 4.2 %; for SEXED and CONV, respectively). Accordingly, previous research reported substantial decreases in pregnancy rates to AI when beef or dairy females are inseminated with sexsorted semen, either at fixed-time AI or upon estrus detection (Sá Filho et al., 2012; Seidel et al., 1999). These outcomes are mostly attributed to sperm damage associated with the sorting and cryopreservation processes, which reduces the viability and quality of the sorted sperm (Seidel, 2007).

Within cows that did not become pregnant to AI, pregnancy rates to bull breeding were similar (P = 0.51) between CONV and SEXED cows (Table 2). Given that the bull to cow ratio was approximately 1:25 (36 bulls and 893 cows exposed in the experiment), and a total of 492 cows did not become pregnant to AI (Table 2), the actual bull to non-pregnant cow ratio was 1:14. Hence the number of bulls available to service non-pregnant cows was above the recommended ratio for a 50day breeding season (Healy et al., 1993; Pexton et al., 1990), which likely contributed to the similar pregnancy rates to bull breeding between CONV and SEXED cows. However, final pregnancy rates (AI + bull breeding) were also reduced (P = 0.05) for SEXED compared with CONV cows (Table 2). Within pregnant cows only, SEXED cows had a reduced (P < 0.01) proportion of pregnancies to AI and hence greater (P < 0.01) proportion of pregnancies to bull breeding compared with CONV cows (Table 2).

Calving results. Within pregnant cows to AI, the proportion of male calves born was greater (P < 0.01) in SEXED compared with CONV cows (Table 2). No differences were detected (P = 0.38) in the proportion of male calves from cows pregnant to bull breeding (Table 2). Accordingly, pregnant SEXED cows also had a greater (P < 0.01) proportion of male calves at the end of the calving season (AI + bull breeding) compared with pregnant CONV cows (Table 2). Calves from SEXED cows had greater (P = 0.05) birth BW compared with calves from CONV cows (Table 3). However, SEXED and CONV cows had similar (P = 0.19) kg of calf born/cow exposed to breeding (Table 3). The proportion of male calves born to AI in SEXED cows was in accordance with the expected male to female ratio yielded by the semen sorting process (Rath and Johnson,

Table 1. Age, BCS, synchronization rate, and pregnancy rates to AI in cows inseminated with sexed (n = 439) or conventional (CONV: n = 454) semen.¹

Item	SEXED	CONV	SEM	<i>P</i> =
Age, yr	5.43	5.47	0.13	0.83
BCS at AI	4.75	4.73	0.03	0.45
Synchronization rate, %	84.6	84.5	1.7	0.96

¹ Within parenthesis, number of cows divided by total cows.

Table 2. Pregnancy rates to AI, bull breeding, final pregnancy rates (AI + bull breeding), and proportion of male calves born from cows inseminated with sexed (n = 439) or conventional (CONV; n = 454) semen.

Item	SEXED	CONV	SEM	P=
Pregnancy rates to AI, %				
All cows	34.9	56.0	2.2	< 0.01
Synchronized cows	40.6	66.1	2.4	< 0.01
Pregnancy rates to bull, %	74.6	72.0	2.8	0.51
Final pregnancy rates,2 %	83.5	87.9	1.6	0.05
Pregnancies to AI, %	41.6	63.8	2.4	< 0.01
Pregnancies to bull, %	58.4	36.2	2.4	< 0.01
Proportion of males calves, 3 %				
Pregnancies to AI,	91.2	57.3	3.0	< 0.01
Pregnancies to bull,	48.2	52.9	3.8	0.38
All pregnancies	65.5	55.3	2.5	< 0.01

¹ Within parenthesis, number of cows divided by total cows. Beginning 18 d after AI, cows were exposed to mature Angus and Hereford bulls for 50 d.

² Within cows classified as pregnant, the proportion of cows pregnant to AI or bull breeding.

³ Proportion of males calves in the calf crop sired by AI (pregnancies to AI), bull breeding (pregnancies to bull breeding), or AI + bull breeding (all pregnancies).

2008), which increased the final proportion of male calves born to SEXED compared with CONV cows during the calving season (Table 2).

Weaning results – calf parameters. No treatment effects were detected ($P \ge 0.31$) for calf loss from birth to weaning and weaning rate (Table 3). The proportion of steers weaned was greater (P < 0.01) in SEXED compared with CONV cows (Table 3).

A treatment × calf gender interaction was detected (P < 0.01) for calf age, BW, and estimated value at weaning. Steers from SEXED cows were younger (P < 0.01) compared with steers from CONV cows (Table 4), whereas no treatment effects were detected for steer BW and estimated value at weaning ($P \ge 0.75$). Heifers from SEXED cows were lighter (P = 0.05), younger (P < 0.01), and had reduced (P < 0.01) estimated value at weaning compared with heifers from CONV cows (Table 4). Across genders, calves from SEXED cows had reduced ($P \le 0.01$) weaning age and BW compared with calves from CONV cows (Table 3), while estimated calf value at weaning did not differ (P = 0.24) between treatments (Table 4).

A treatment × calf gender interaction was also detected (P < 0.01) for 205-d adjusted BW and subsequent estimated weaning value. Heifers from SEXED cows also had reduced 205-day adjusted weaning BW (P < 0.01) and estimated weaning value (P = 0.01) compared with heifers from CONV

Table 3.	. Calf and cow-calf	performance	parameters	from	cows	inseminated	with	sexed	(n = 439)	or conventional	(CONV; n	= 454)
semen.1												

Item	SEXED	CONV	SEM	P-value
Calf parameters				
Birth BW, kg	40.4	39.6	0.3	0.05
Weaning age, kg	206.4	212.6	0.9	< 0.01
Weaning BW, kg	239.3	245.6	1.8	0.01
205-day adjusted weaning BW,2 kg	246.4	247.5	1.3	0.56
Cow-calf production parameters ³				
Kg of calf born per cow exposed to breeding, kg	33.8	35.1	0.7	0.19
Calf loss from birth to weaning, %	4.8 (17/367)	6.5 (27/400)	1.2	0.31
Weaning rate, %	79.5 (350/439)	82.0 (373/454)	1.8	0.34
Proportion of steers weaned, %	66.1 (232/350)	56.2 (210/373)	2.5	< 0.01
Kg of calf weaned/cow exposed to breeding, kg				
Steers, kg	130.4	115.2	6.0	0.05
Heifers, kg	59.5	85.8	5.1	< 0.01
Overall	189.9	201.0	4.7	0.09
205-day adjusted kg of calf weaned/cow exposed to breeding,2 kg				
Steers, kg	133.1	116.7	6.1	0.05
Heifers, kg	62.4	86.4	5.2	< 0.01
Overall	195.5	203.2	6.7	0.25

¹ Within parenthesis, number of cows divided by total cows. ² Calculated according to BIF (2010). ³ Kilograms of calf born and calf weaned per cow exposed to breeding were calculated based on calving rate, weaning rate, and calf BW at birth and weaning.

Item	SEXED	CONV	SEM	P-value
Original calf weaning BW				
Weaned steers				
Age at weaning, d	210.6	213.0	0.5	< 0.01
Weaning BW, kg	250.3	250.9	2.1	0.84
Calf value, US\$	641.6	643.3	3.8	0.75
Weaned heifers				
Age at weaning, d	197.7	212.3	1.4	< 0.01
Weaning BW, kg	218.3	238.4	2.5	< 0.01
Calf value, US\$	529.1	565.3	4.6	< 0.01
Overall				
Value per calf, US\$	603.2	609.5	3.8	0.24
Calf value/cow exposed to breeding, US\$	479.0	499.3	11.7	0.22
205-day adjusted calf weaning BW ²				
Weaned steers				
Weaning BW, kg	253.7	253.5	1.7	0.95
Calf value, US\$	646.3	645.2	2.8	0.78
Weaned heifers				
Weaning BW, kg	232.4	239.7	1.8	< 0.01
Calf value, US\$	553.6	564.7	3.1	0.01
Overall				
Calf value, US\$	614.7	610.0	3.1	0.27
Calf value/cow exposed to breeding, US\$	488.3	500.7	11.6	0.45

Table 4. Estimated weaning economical returns, based on original or 205-day adjusted calf weaning BW, from cows inseminated with sexed (n = 439) or conventional (CONV; n = 454) semen.¹

¹ Within parenthesis, number of cows divided by total cows. ² Calculated according to BIF (2010).

cows, whereas these parameters were similar ($P \ge 0.78$) among steers from CONV and SEXED cows (Table 4). Across genders, calves from SEXED cows had similar 205-day adjusted BW (P = 0.25; Table 3) and estimated weaning value compared with calves from CON cows (P = 0.27; Table 4).

Cow-calf production parameters. A treatment × calf gender interaction was detected (P < 0.01) for kg of calf weaned/cow exposed to breeding. Cows assigned to SEXED had greater (P = 0.05) kg of steer weaned/cow exposed to breeding, but reduced kg of heifer weaned/cow exposed to breeding (P < 0.01) compared with CONV cows (Table 3). Across genders, SEXED cows tended (P = 0.09) to have reduced kg of calf weaned/cow exposed to breeding compared with CONV cows (Table 3), whereas estimated calf value/cow exposed to breeding did not differ between treatments (P = 0.22 Table 4).

A treatment × calf gender interaction was also detected (P < 0.01) for 205-day adjusted kg of calf weaned/cow exposed to breeding. Cows assigned to SEXED had greater (P = 0.05) 205-day adjusted kg of steer weaned/cow exposed to breeding, but reduced 205-day adjusted kg of heifer weaned/ cow exposed to breeding (P < 0.01) compared with CONV cows (Table 3). Across genders, no treatment effects were detected ($P \ge 0.25$) for 205-day adjusted kg of calf weaned/ cow exposed to breeding (Table 3) or estimated calf value/ cow exposed to breeding (Table 4).

It is important to note that this experiment did not account for any additional costs associated with purchasing sexed semen (Seidel, 2007), which may impact the economical returns of cows inseminated with sexed-semen. Nevertheless, results from this experiment suggest that inseminating beef cows with sexed semen does not improve economic returns in cow-calf operations that market the calf crop upon weaning.

IMPLICATIONS

Inseminating beef cows with sexed semen reduced pregnancy rates to AI and final pregnancy rates (AI + 50day bull breeding), but increased the proportion of steers weaned and kg of steers weaned/cow exposed to breeding. However, overall kg of calf weaned/cow exposed to breeding and estimated calf value/cow exposed to breeding were not improved by the use of sexed semen, particularly because of its negative impacts on weaning age and BW of the heifer progeny. Based on these results, inseminating beef cows with sexed semen may not be a viable option to improve economic returns in cow-calf systems that inseminate and expose the cowherd to a 50-day bull breeding, and subsequently market the calf crop upon weaning.

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Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: I. Performance, reproductive, and metabolic responses of replacement heifers

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INTRODUCTION

ABSTRACT: This experiment evaluated the influence of supplement composition on performance, reproductive, and metabolic responses of Angus × Hereford heifers consuming a low-quality cool-season forage (8.7 % CP and 57 % TDN). Sixty heifers were allocated into 15 drylot pens (4 heifers/pen; 5 pens/treatment), and assigned to: 1) supplementation with soybean meal (PROT), 2) supplementation with a mixture of cracked corn, soybean meal, and urea (68:22:10 ratio, DM basis; ENER), or 3) no supplementation (CON). Heifers received meadow foxtail hay for ad libitum consumption throughout the experiment (d -10 to 160). Beginning on d 0, PROT and ENER were provided daily at 1.30 and 1.40 kg of DM/heifer to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Hay and total DMI were recorded monthly for 5 consecutive days. Blood was collected every 10 d for analysis of plasma progesterone to evaluate puberty attainment. Blood samples collected on d -10, 60, 120, and 150 were also analyzed for plasma urea N (PUN), glucose, insulin, IGF-I, NEFA, and leptin. Liver samples were collected on d 100 from 2 heifers/ pen, and analyzed for mRNA expression of genes associated with nutritional metabolism. No treatment effect was detected (P = 0.33) on hay DMI. Total DMI, ADG, mean concentrations of glucose, insulin, and IGF-I, and hepatic mRNA expression of IGF-I and IGFBP-3 were greater ($P \le 0.02$) for PROT and ENER compared with CON, and similar between PROT and ENER ($P \ge 0.13$). Mean PUN was greater (P < 0.01) for PROT and ENER compared with CON, whereas PROT heifers had greater (P < 0.01) PUN compared with ENER. Plasma leptin concentrations were similar between ENER and PROT ($P \ge 0.19$), and greater ($P \le 0.03$) for ENER and PROT compared with CON on d 120 and 150 (P = 0.03). Hepatic mRNA expression of mitochondrial phosphoenolpyruvate carboxykinase was greater (P = 0.05) in PROT compared with CON and ENER, and similar between CON and ENER (P = 0.98). The proportion of heifers pubertal on d 160 was greater (P < 0.01) in ENER compared with PROT and CON, and similar between PROT and CON (P = 0.38). In conclusion, beef heifers consuming a low-quality cool-season forage had a similar increase in DMI, growth, and overall metabolic status if offered supplements based on soybean meal or corn at 0.5 % of BW.

Keywords: Beef heifers, gene expression, low-quality coolseason forage, metabolism, performance, supplementation

Supplementation is often required in heifer development programs based on low-quality forages. Although forages typically represent the main source of energy for foragefed cattle, and energy is the primary dietary consideration for heifer development, protein is traditionally considered the limiting nutrient in Western U.S. cow-calf operations (DelCurto et al., 2000). Indeed, protein supplementation generally improves digestibility and DMI of low-quality warm-season forages, resulting in increased energy utilization from the forage and cattle BW gain (DelCurto et al., 1990). However, Bohnert et al. (2011) reported that protein supplementation did not increase digestibility and DMI of low-quality cool-season forages. Hence, inclusion of energy ingredients into supplements may be required for optimal growth and reproductive development of replacement heifers consuming low-quality cool-season forages.

Beef heifers, particularly Bos taurus, should attain puberty by 12 mo of age to maximize their lifetime productivity (Lesmeister et al., 1973). Energy intake also influences puberty attainment in heifers by other mechanisms besides BW gain, including modulation of hormones known to mediate puberty, such as insulin and IGF-I. Accordingly, Ciccioli et al. (2005) reported that feeding starch-based supplements hastened puberty attainment in beef heifers independently of BW gain. Hence, inclusion of energy ingredients, such as starch, into supplements may further benefit reproductive development of heifers consuming low-quality cool-season forages by favoring circulating concentrations of nutritional mediators of puberty. To test this hypothesis, this experiment compared the effects of supplements based on protein or energy ingredients on performance, plasma metabolites and hormones, expression of hepatic genes associated with nutritional metabolism, and puberty attainment of beef heifers consuming a low-quality cool-season forage.

MATERIALS AND METHODS

Heifers and diets. Sixty Angus \times Hereford weaned heifers (initial age 226 ± 3 d; initial BW 200 ± 2 kg) were used. On d -10 of the study, heifers were ranked by initial BW and age and allocated to 15 drylot pens (5 pens/treatment; 4 heifers/pen), in a manner which all pens had equivalent initial average BW and age. Pens were randomly assigned to receive 1 of 3 treatments: 1) supplementation with soybean [Glycine max (L.) Merr.] meal (PROT), 2) supplementation with a mixture of cracked corn (Zea mays L.), soybean meal, and urea (68:22:10 ratio, DM basis; ENER), or 3) no supplementation (CON). Heifers were offered meadow foxtail (Alopecurus pratensis L.) hay for ad libitum consumption during the entire experiment (d -10 to 160). Beginning on d 0, PROT and ENER treatments were fed once daily (0800 h) at a rate of 1.30 and 1.40 kg of DM/heifer, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous (Table 1). Urea was included into ENER to result in isocaloric and isonitrogenous intakes of PROT and ENER. Further, treatment intakes were formulated at 0.50 and 0.54 % of the expected average heifer shrunk BW during the experiment for PROT and ENER, respectively. Average heifer shrunk BW during the experiment was estimated based on initial shrunk BW (d -9) and expected final shrunk BW (d 161). Expected final shrunk BW was projected based on previous research from our group (Cooke et al., 2013), which was conducted at the same research station and using the same cowherd as the experiment described herein.

Sampling. Heifers were weighed on 2 consecutive d to determine both full and shrunk (after 16 h of feed and water restriction) BW at the beginning (d -10 and -9) and end of the study (d 160 and 161). Shrunk BW was used to determine heifer ADG during the study. Blood samples were collected at 10-d intervals throughout the entire experiment (d -10 to 160), starting 4 h after the ENER and PROT treatments were offered, to determine onset of puberty according to plasma progesterone $(\mathbf{P}_{\mathbf{A}})$ concentration. Heifers were considered pubertal when plasma P₄ concentration was equal or greater than 1.0 ng/mL for 2 consecutive samplings (Perry et al., 1991), and puberty attainment was declared at the second sampling of elevated progesterone. Blood samples collected on d -10, 60, 120, and 150 were also analyzed for plasma urea N (PUN), glucose, insulin, NEFA, IGF-I, and leptin concentrations. Samples were processed and analyzed according to procedures described by Cooke et al. (2012).

Hay and total DMI were evaluated from each pen by collecting and weighing refusals from d 12 to 16, d 53 to 57, d 71 to 75, d 93 to 97, d 112 to 116, and d 143 to 147 of the experiment, which were classified as periods (periods 1 to 6, respectively). Samples of the offered and non-consumed hay were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of heifers within each pen and expressed as kg per heifer/d. Daily intake of NE_m , NE_g , CP, RDP, and starch were estimated based on DMI of each pen, and nutritive value of hay and treatments (Table 1).

On d 100 of the experiment, 2 heifers/pen were randomly assigned for liver sample collection via needle biopsy (Cooke et al., 2008), which began 4 h after supplements were offered. Samples were processed and analyzed via real-time quantitative reverse transcription (**RT**)-PCR for IGF-I, IGFBP-3, pyruvate carboxylase (**PC**), cytosolic phosphoenolpyruvate carboxykinase (**PEPCK-C**), mitochondrial PEPCK (**PEPCK-M**), and cyclophilin mRNA expression, according to Cooke et al. (2008) and Yoganathan et al. (2012).

Statistical analysis. All data were analyzed using pen as experimental unit, and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Performance, plasma variables, and gene expression data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model statement used for BW, ADG, and gene expression contained only the effects of treatment. Data were analyzed using heifer(pen) and pen(treatment) as the random variables. The model statement used for plasma variables contained the effects of treatment, day, the treatment \times day interaction, and values obtained on d -10 as covariate. Data were analyzed using heifer(pen) and pen(treatment) as random variables, with day as the specified term for the repeated statement and heifer(pen) as subject. The model statement used for feed and nutrient intake contained the effects of treatment, day, period, and all the resultant interactions. Data were analyzed using pen(treatment) as the random variable, given that DMI was recorded daily from each pen, as well as day(period) as the specified term for the repeated statement and pen(treatment) as subject. For both intake and plasma variables, the covariance structure used was first-order autoregressive, which provided the smallest Akaike Information Criterion and hence the best fit for all variables analyzed. Puberty data were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc.). The model statement used contained the effects of treatment, day, and the resultant interaction. Data were analyzed using heifer(pen) and pen(treatment) as the random variables. Results are reported as least square means, or covariately adjusted means for plasma variables, and separated using PDIFF. Significance was set at $P \le 0.05$ and tendencies were denoted if P > 0.05and ≤ 0.10 . Results are reported according to main effects if no interactions were significant, or according to highestorder interaction detected.

RESULTS AND DISCUSSION

No treatment effects were detected (P = 0.33) on forage DMI (Table 2). These results support that protein supplementation does not impact DMI of a low-quality coolseason forage (Bohnert et al., 2011), and that supplements based on energy ingredients can be fed at approximately 0.5 % of BW without impacting forage intake (Bowman and Sanson, 1996). Total daily DMI, and estimated daily intake of NE_m, and NE^g were greater (P < 0.01) for PROT and ENER compared with CON, and similar ($P \ge 0.41$) between PROT and ENER (Table 2). Estimated daily intake of CP, RDP, and starch were greater (P < 0.01) for PROT and ENER compared with CON, whereas ENER had greater (P < 0.01) RDP and starch intake, and tended (P = 0.09) to have less CP intake compared to PROT heifers (Table 2). Hence, PROT and ENER had greater overall nutrient intake compared with CON heifers. The greater RDP intake of ENER compared with PROT heifers can be attributed to the inclusion of urea into the ENER treatment, and consequent RDP content of treatments (Table 1). In addition, the slightly greater CP intake of PROT compared with ENER, despite similar CP content of treatments can be attributed to the numerical difference in hay intake between PROT and ENER.

A treatment effect (P < 0.01) was detected for ADG (Table 2), which was greater (P < 0.01) for PROT and ENER compared with CON, and similar between ENER and PROT (P = 0.52). These results provide evidence that beef heifers consuming low-quality cool-season forages can equally utilize nutrients provided by supplements based on protein or energy ingredients to support BW gain. Furthermore, differences in CP and RDP intakes between ENER and PROT were minimal and not sufficient to impact heifer ADG.

A treatment effect was detected (P < 0.01) for plasma NEFA (Table 3). Mean NEFA concentration was greater (P < 0.01) for CON compared with PROT and ENER, and similar (P = 0.13) between PROT and ENER. Accordingly, circulating NEFA in cattle was negatively associated with nutrient intake and ADG. However, it is important to note that elevated NEFA is often associated with negative energy balance (Lucy et al., 1991), and heifers from all treatments were in a positive nutritional status based on their ADG (Table 2).

A treatment effect was detected (P < 0.01) for PUN (Table 3). During the study, mean PUN was greater (P < 0.01) for PROT and ENER compared with CON, whereas PROT also had greater (P < 0.01) PUN compared with ENER (Table 3). The greater PUN concentrations of PROT and ENER compared with CON can be directly attributed to their greater CP and RDP intake, and suggest that CON heifers required supplemental CP and RDP. Differences in PUN between ENER and PROT heifers can also be attributed to the slightly greater CP intake of PROT heifers, as well as improved N utilization by ruminal microbes in ENER heifers (Hall and Huntington, 2008).

Mean glucose concentration was greater (P < 0.01) for PROT and ENER compared with CON, and similar (P=0.91) between PROT and ENER (Table 3). In agreement, glucose concentration was positively associated with feed intake and BW gain (Vizcarra et al., 1998), as observed herein based on the greater nutrient intake and ADG of PROT and ENER heifers. However, starch is the major dietary precursor for glucose in ruminants; hence, it would be expected that ENER heifers had greater plasma glucose compared to PROT. Nevertheless, Huntington (1997) reported that growing cattle are capable of synthesizing glucose from amino acids.

Supporting this latter rationale, PROT heifers had greater (P = 0.05) mRNA expression of liver PEPCK-M compared with ENER and CON, which was similar (P = 0.98) between ENER and CON (Table 4). No treatment effects were detected ($P \ge 0.28$; Table 4) for mRNA expression of PC and PEPCK-C, although mRNA expression of these enzymes are modulated by nutrient intake (Cooke et al., 2008) and positively associated with glucose synthesis in cattle (Bradford and Allen, 2005). Nevertheless, circulating NEFA stimulate mRNA expression of PC and PEPCK-C,

Table 1. Ingredient composition and nutrient profile of treatments offered during the experiment.

Item	PROT	ENER
Ingredients, % DM		
Cracked corn		68
Soybean meal	100	22
Urea		10
Nutrient profile, DM basis		
TDN, %	85.4	77.0
NE _m , Mcal/kg	2.02	1.91
NEg, Mcal/kg	1.37	1.31
CP, %	50.1	45.0
RDP, %	28.3	36.0
NFC, %	33.5	59.0
NDF, %	8.6	9.0
Starch, %	5.4	48.4
Ether extract, %	1.5	2.9
Daily intake		
DM, kg	1.30	1.40
TDN, kg	1.11	1.08
NE _m , Mcal	2.63	2.67
NEg, Mcal	1.78	1.83
CP, kg	0.65	0.63
RDP, kg	0.37	0.50
NFC, kg	0.44	0.83
NDF, kg	0.11	0.13
Starch, kg	0.07	0.68
Ether extract, kg	0.02	0.04

Table 2. Performance and puberty parameters of replacement of replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (CON; n = 5), or supplements based on protein (**PROT**; n = 5) or energy ingredients (**ENER**; n = 5).¹

Item	CON	PROT	ENER	SEM	P =
DMI, kg/d					
Hay	5.94	5.79	5.51	0.20	0.33
Total	5.94	7.10 ^b	6.91 ^b	0.19	< 0.01
Daily nutrient intake					
NE _m , Mcal	6.54ª	9.00 ^b	8.74 ^b	0.22	< 0.01
NEg, Mcal	3.27ª	4.97 ^b	4.87 ^b	0.11	< 0.01
CP, kg	0.51 ⁿ	1.15 ^b	1.11 ^b	0.02	< 0.01
RDP, kg	0.35ª	0.71 ^b	0.83°	0.01	< 0.01
Starch, kg	0.10 ^a	0.17 ^b	0.77°	0.003	< 0.01
ADG, kg/d	0.36 ^a	0.76 ^b	0.72 ^b	0.04	< 0.01
Puberty on d 160, %	10 ^a	5ª	25 ^b	4	< 0.01

¹ Within rows, values with different superscripts differ ($P \le 0.10$).

but no PEPCK-M, to preserve gluconeogenesis in cattle with insufficient nutrient intake (White et al., 2011). Hence, the greater NEFA concentration in CON heifers likely maintained mRNA expression of PC and PEPCK-C similar to that of ENER and PROT.

Treatment effects were detected ($P \le 0.05$) for plasma insulin and IGF-I (Table 3), as well as mRNA expression of liver IGF-I and IGFBP-3 (Table 4). Mean insulin and IGF-I

Table 3. Plasma concentrations of NEFA, urea N (**PUN**), glucose, insulin, and IGF-I of replacement beef heifers consuming a lowquality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 5), or supplements based on protein ingredients (**PROT**; n = 5) or energy ingredients (**ENER**; n = 5).¹

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Item	CON	PROT	ENER	SEM	P =
NEFA, µEq/L	0.412 ^a	0.194 ^b	0.241 ^b	0.022	< 0.01
PUN, mg/dL	3.57 ^a	20.07 ^b	17.87 ^c	0.54	< 0.01
Glucose, mg/dL	59.3ª	65.1 ^b	65.0 ^b	1.1	< 0.01
Insulin, µIU/mL	5.20 ^a	6.72 ^b	6.69 ^b	0.35	0.02
IGF-I, ng/mL	79.5 ^a	159.4 ^b	149.5 ^b	5.5	< 0.01

¹ Within rows, values with different superscripts differ ($P \le 0.10$).

Table 4. Expression of hepatic genes associated with nutritional metabolism of replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (CON; n = 5), or supplements based on protein ingredients (**PROT**; n = 5) or energy ingredients (**ENER**; n = 5).¹

Item	CON	PROT	ENER	SEM	P =
PC	3.64	2.77	2.66	0.45	0.28
PEPCK-C	5.00	4.68	3.92	0.68	0.52
PEPCK-M	2.92 ^a	4.19 ^b	2.90^{a}	0.42	0.08
IGF-I	3.71 ^a	8.31 ^b	6.75 ^b	1.00	0.02
IGFBP-3	1.62 ^a	2.46 ^b	2.38 ^b	0.21	0.03

¹ Within rows, values with different superscripts differ ($P \le 0.10$).

concentrations were greater (P < 0.01) for PROT and ENER compared with CON, and similar ($P \ge 0.21$) between PROT and ENER (Table 3). Expression of liver IGF-I and IGFBP-3 mRNA were also greater ($P \le 0.05$) in PROT and ENER compared with CON, and similar ($P \ge 0.29$) between PROT and ENER (Table 4). These results corroborate with treatment effects detected for DMI, nutrient intake, and plasma glucose, given that circulating concentration of insulin is positively regulated by nutrient intake and blood glucose (Vizcarra et al., 1998). Availability of energy substrates and circulating insulin positively modulate the expression of liver IGF-I and IGFBP-3 mRNA, and consequent hepatic synthesis of these proteins (Cooke et al., 2008). For these reasons, plasma insulin and IGF-I have been recognized as indicators of nutritional status of cattle (Hess et al., 2005).

A treatment × day interaction was detected (P = 0.03) for plasma leptin (Figure 1). Plasma leptin concentrations were similar between ENER and PROT throughout the experiment ($P \ge 0.19$), and greater for ENER and PROT compared with CON on d 120 ($P \le 0.01$) and 150 ($P \le 0.03$; Figure 1). Circulating leptin is regulated by body fat content, nutrient intake, and circulating insulin (Houseknecht et al., 1998). Nevertheless, the greater ADG, nutrient intake, and plasma insulin of PROT and ENER heifers compared with CON only resulted in a similar effect on plasma leptin beginning on d 120 of the experiment. The reason for this delay is unknown and deserves further investigation, but may be associated with heifer age and rate of body fat accretion (Houseknecht et al., 1998).



Figure 1. Plasma concentrations of leptin in replacement beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 5), or supplements based on protein (**PROT**; n = 5) or energy (**ENER**; n = 5) ingredients. A treatment × hour interaction was detected (P < 0.01). Within day, letters indicate differences between treatments ($P \le 0.03$).

No treatment effects were detected (P = 0.25) on puberty attainment (data not shown). However, a greater (P < 0.01) proportion of ENER heifers were pubertal at the end of the experiment (d 160) compared with CON and PROT, whereas no differences were detected (P = 0.38) between CON and PROT (Table 2). The main hypothesis of the experiment was that replacement beef heifers consuming a low-quality coolseason forage and receiving a supplement based on an energy ingredient would have hastened puberty attainment compared with heifers receiving no supplementation or supplemented with a protein ingredient. This hypothesis was developed based on the premise that energy sources such as corn favor circulating concentrations of insulin, IGF-I, and leptin (Lents et al., 2005), which impact the puberty process by mediating synthesis and activity of GnRH and gonadotropin (Maciel et al., 2004). Indeed, a greater proportion of ENER heifers were pubertal at the end of experiment compared with PROT and CON, despite the similar ADG and metabolic status between PROT and ENER. Likewise, Ciccioli et al. (2005) reported that heifers receiving a high-starch supplement had hastened puberty attainment but similar ADG compared with cohorts receiving a low-starch supplement. However, these results should be interpreted with caution, because overall puberty attainment herein was lower than expected based on previous work from our research group (Cooke et al., 2012; 2013). The reason for this outcome is unknown, given that ENER and PROT heifers achieved the BW recommended for puberty achievement at 13 mo of age (Patterson et al., 2000). More specifically, % mature BW on d 160 was greater (P < 0.01) for ENER and PROT compared to CON (50.7, 62.6, and 65.1 % of mature BW, for CON, ENER, and PROT, respectively), whereas heifer age on d 160 was similar among treatments (P = 0.97) and averaged 396 ± 6 d.

IMPLICATIONS

Replacement beef heifers offered PROT and ENER had a similar increase in nutrient intake, ADG, and overall metabolic status compared with CON heifers, despite differences in ingredients between treatments. Puberty attainment was enhanced in ENER heifers only, although this outcome should be interpreted with caution due to the reduced number of pubertal heifers across all treatments. Hence, replacement beef heifers consuming a low-quality cool-season forage can equally utilize and benefit, in terms of growth and metabolic parameters, from supplements based on protein or energy ingredients provided as 0.5 % of heifer BW/d at isocaloric and isonitrogenous rates.

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Effects of meloxicam administration on physiological and performance responses of transported feeder cattle

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ABSTRACT: Eighty-four Angus × Hereford steers were ranked by BW on d -10, and assigned to 21 drylot pens. From d -10 to 0, pens were fed alfalfa-grass hay ad libitum and 2.4 kg/steer daily (DM basis) of a corn-based concentrate. On d 0, pens were randomly assigned to transport for 1,440 km in a livestock trailer and receive meloxicam (MEL; 1 mg/ kg of BW daily; n = 7) or lactose monohydrate (TRANS; 1 mg/kg of BW daily; n = 7) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving, or no transport receiving lactose monohydrate concurrently with treatment administration to MEL and TRANS (CON; 1 mg/kg of BW daily; n = 7). Upon arrival (d 1), MEL and TRANS steers returned to their original pens for a 21-d feedlot receiving with the same pre-transport diet. Treatments were administered via individual oral drench on d 0 and 1, or mixed with the concentrate from d 2 to 7. Full BW was recorded prior to (d -2, -1, and 0) transport and at the end of experiment (d 20, 21, and 22) for ADG calculation. Daily DMI was recorded from d 1 to 21. Blood samples were collected on d 0, 1, 3, 5, 7, 10, 14, and 21. During the initial 7 d of feedlot receiving, hay and total DMI were reduced ($P \le 0.03$) in TRANS vs. CON and MEL, similar between CON and MEL ($P \ge 0.26$), whereas concentrate DMI did not differ (P = 0.16) among treatments. Mean ADG and G:F were reduced ($P \le 0.03$) in TRANS vs. MEL and CON, but similar ($P \ge 0.39$) between MEL and CON. Serum NEFA concentrations were greater (P < 0.01) for TRANS and MEL vs. CON on d 1. Plasma haptoglobin concentrations were greater ($P \le 0.03$) for TRANS vs. CON and MEL on d 5, and greater ($P \le 0.03$) for CON vs. TRANS on d 10. Plasma ceruloplasmin concentrations were greater $(P \le 0.04)$ for TRANS vs. CON on d 3, 5, 7, 10, and 14, greater ($P \le 0.03$) for TRANS vs. MEL on d 5 and 7, and also greater (P = 0.05) for MEL vs. CON on d 3. Hence, meloxicam administration to feeder steers reduced the acutephase protein response and prevented the performance losses caused by long-distance transportation.

Keywords: Acute-phase proteins, cattle, feedlot, meloxicam, transport

INTRODUCTION

Road transport is one of the most stressful events encountered by feeder cattle during their productive lives. Upon long-distance transportation and feedlot arrival, cattle experience inflammatory and acute-phase responses (Arthington et al., 2008; Cooke et al., 2011) that impact feedlot receiving performance by increasing basal metabolism, tissue catabolism, and by reducing DMI and G:F (Johnson, 1997). Hence, strategies that lessen the acute-phase response during feedlot receiving, which can be monitored via acute-phase proteins such as haptoglobin and ceruloplasmin (Carroll and Forsberg, 2007), improve productivity of transported cattle (Arthington et al., 2008).

Administration of flunixin meglumine to steers prior to a 24-h road transport and at feedlot arrival alleviated the resultant acute-phase response but did not improve feedlot receiving performance (Cooke et al., 2013a). Perhaps the elimination half-life of flunixin meglumine (less than 8 h; Odensvik and Johansson, 1995) was insufficient to modulate the transport-elicited acute-phase response to an extent that resulted in enhanced cattle performance. Alternatively, meloxicam has an elimination half-life of 28 h (Coetzee et al., 2009) when orally administered to cattle at 1 mg/kg. Accordingly, Van Engen et al. (2014) reported that oral administration of meloxicam to cattle prior to a 16-h road transport reduced transport-induced inflammatory reactions, although authors did not evaluate feedlot receiving performance. Based on this rationale, we hypothesized that oral meloxicam administration prior to transport and during feedlot receiving alleviates the acute-phase response and improves performance of feeder cattle. Hence, the objective of this experiment was to evaluate the effects of oral meloxicam administration on circulating concentrations of cortisol, NEFA, acute-phase proteins, and feedlot receiving performance of transported cattle.

MATERIALS AND METHODS

Animals and diets. Eighty-four Angus × Hereford steers, weaned 40 d prior to the beginning of the experiment (d -10), were utilized. On d -10, steers were ranked by BW (252 ± 3 kg; initial age 214 ± 2 d) and randomly allocated to 21 dry lot pens (4 steers/pen; 7×15 m) in manner which all pens had equivalent average BW. From d -10 to 0, all pens were fed alfalfa-grass hay ad libitum and 2.4 kg/steer daily (DM basis) of a concentrate containing (as-fed basis) 84% cracked corn, 14% soybean meal, and 2% mineral mix, which was offered separately from hay at 0800 h. On d 0, pens were randomly assigned to 1 of 3 treatments: 1) transport for 1,440 km in a commercial livestock trailer and oral administration of meloxicam (1 mg/kg of BW; Carlsbad Technologies, Inc., Carlsbad, CA) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving (**MEL**; n = 7), 2) transport for 1,440 km in a commercial livestock trailer and oral administration of lactose monohydrate (1 mg/ kg of BW, excipient used in the manufacture of meloxicam tablets; Avantor Performance Materials, Center Valley, PA) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving (**TRANS**; n = 7), or 3) no transport and oral administration of lactose monohydrate (1 mg/kg of BW; Avantor Performance Materials) concurrently with treatment administration to MEL and TRANS steers (**CON**; n = 7).

On d 0 of the experiment. MEL and TRANS steers were commingled and transported at the same time and in the same double-deck commercial livestock trailer (Legend 50' cattle liner; Barrett LLC., Purcell, OK), while CON steers remained in their respective drylot pens with ad libitum access to alfalfa-grass hay and 2.4 kg/steer (DM basis) of the aforementioned concentrate. Upon arrival (d 1), MEL and TRANS steers returned to their original pens for a 21-d feedlot receiving. All pens were fed alfalfa-grass hay ad libitum and 2.4 kg/steer daily (DM basis) of the aforementioned corn-based concentrate during the receiving period, which was offered separately from hay at 0800 h. Water was offered for ad libitum consumption from d -10 to 28, except to MEL and TRANS cattle during transport.

Meloxicam was originally presented in 15 mg tablets, which were ground daily using a commercial food processor (Soho Food Processor; West Bend Housewares, West Bend, WI) to ensure that MEL steers received their exact dose. Lactose monohydrate was administered to TRANS and CON steers to account for potential placebo effects, whereas the CON treatment was included as a non-transport positive control for physiological and performance measurements. On d 0 and 1, meloxicam or lactose monohydrate were manually mixed with 50 mL of 0.9% saline and administered individually to steers via oral drench during handling of MEL and TRANS for truck loading (d 0) or feedlot arrival (d 1). Treatments were mixed with saline within 30 s prior to administration. From d 2 to 7, treatments were mixed daily with the corn-based concentrate according to the total BW of each pen.

Sampling. Individual full BW was recorded and averaged over 3 consecutive days prior to treatment application (d -2, -1, and 0) and at the end of experiment (d 20, 21, and 22) for ADG calculation. Average BW of d -2, -1, and 0 was used to determine meloxicam and lactose monohydrate doses. Individual BW was also collected on d 1, immediately prior treatment application, to evaluate BW shrink as percentage change from the average BW recorded on d -2, -1, and 0. Concentrate, hay, and total DMI were evaluated daily from d -10 to 21 from each pen by collecting and weighing refusals daily. Samples of the offered and non-consumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of steers within each pen, and expressed as kg per steer/d. Total BW gain and DMI of each pen from d 1 to 21 were used for feedlot receiving G:F calculation.

Blood samples were collected on d 0 and 1 immediately before treatment application, and on d 3, 5, 7, 10, 14, and 21 via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with or without 158 USP units of freeze-dried sodium heparin for plasma and serum collection, respectively. Blood samples were collected prior to concentrate feeding, except for d 0 when MEL and TRANS cattle were transported after blood collection. All blood samples were placed immediately on ice, centrifuged (2,500 \times g for 30 min; 4°C) for plasma or serum harvest, and stored at -80°C on the same day of collection. Plasma concentrations of cortisol, NEFA, haptoglobin, and ceruloplasmin were determined as in Cooke et al., (2013a). The intra- and inter-assay CV were, respectively, 3.8 and 3.4% for cortisol, 4.3 and 6.5% for NEFA, 9.1 and 9.0% for ceruloplasmin, and 6.9 and 7.9% for haptoglobin.

Statistical analysis. Data were analyzed using pen as the experimental unit, with the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for BW shrink from d 0 to d 1 and ADG contained the effects of treatment. Data were analyzed using pen(treatment) and steer(pen) as random variables. The model statement used for DMI and G:F contained the effects of treatment, in addition to day, the treatment × day interaction, and average feed intake from d -10 to -1 as covariate for DMI only. Data were analyzed using pen(treatment) as the random variable because DMI was recorded from each pen. The model statement used for blood variables contained the effects of treatment, day, the treatment \times day interaction, and values obtained on d 0 as covariate. Data were analyzed using steer(pen) and pen(treatment) as random variables. The specified term for the repeated statements was day, with pen(treatment) or steer(pen) as subject for DMI or blood variables, respectively. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables analyzed. Results are reported as least square means, as well as covariately adjusted least square means for DMI and blood variables, and were separated using PDIFF. Significance was set at $P \le 0.05$ and tendencies were determined if P > 0.05 and ≤ 0.10 . Results are reported according to treatment effect if no interactions were significant, or according to the highestorder interaction detected that contained the treatment effect.

RESULTS AND DISCUSSION

A treatment effect was detected (P < 0.01) for BW shrink from d 0 to 1. As expected, BW shrink was greater (P < 0.01) for both TRANS and MEL compared with CON steers, and similar (P = 0.14) between TRANS and MEL steers (Table 1). Previous research from our group reported equivalent BW shrink in feeder cattle exposed to the same transportation schedule adopted herein (Marques et al., 2012; Cooke et al., 2013b). No treatment effects were detected ($P \ge 0.13$) on hay, concentrate, and total DMI during the 21-d feedlot receiving (Table 1). Although treatment \times day interactions were also not detected for intake parameters ($P \ge 0.15$), TRANS had reduced hay and total DMI during the initial 7 d of feedlot receiving compared with CON ($P \le 0.03$) and MEL ($P \le 0.01$) steers (5.43, 5.36, and 5.05 kg/steer daily of hay DMI, SEM = 0.09, and 7.85, 7.71, and 7.40 kg/steer daily of total DMI, SEM = 0.09; respectively). Moreover, hav and total DMI during the initial 7 d of feedlot receiving were similar between MEL and CON steers ($P \ge 0.26$), whereas concentrate intake during this period did not differ (P = 0.16) among treatments (2.39, 2.37, and 2.36 kg/steer daily for CON, MEL, and TRANS steers, respectively; SEM = 0.02). No treatment effects were detected for hay, concentrate, and total DMI during the second ($P \ge 0.42$) and third ($P \ge$ 0.28) weeks of feedlot receiving (data not shown). These results indicate that all pens readily consumed their daily concentrate allocation, hence their designed meloxicam and lactose monohydrate dose, during the initial 7 d of feedlot receiving. These results also suggest that oral meloxicam administration prevented the decrease in feed intake often observed in transported cattle during the first week of feedlot receiving (Hutcheson and Cole, 1986; Araujo et al., 2010).

A treatment effect was detected (P = 0.04) for ADG during the 21-d feedlot receiving (Table 1). Steers assigned to TRANS had reduced ADG compared with MEL (P = 0.03) and CON (P=0.01) steers, whereas ADG was similar between (P = 0.82) CON and MEL steers. However, treatment effects detected on ADG were not sufficient to impact (P = 0.78) cattle BW at the end of the 21-d feedlot receiving (Table 1). Nevertheless, a treatment effect was detected (P = 0.03) for G:F during the 21-d feedlot receiving because TRANS had reduced G:F compared with MEL (P = 0.05) and CON steers (P = 0.01), whereas G:F was similar (P = 0.39) between MEL and CON steers (Table 1). Hence, feedlot receiving performance of MEL was similar to CON and greater than TRANS steers, indicating that oral meloxicam administration prevented the performance losses typically observed in cattle transported for long-distances (Hutcheson and Cole, 1986; Marques et al., 2012; Cooke et al., 2013b).

No treatment effect was detected (P = 0.89) for plasma cortisol concentrations during the 21-d feedlot receiving (21.1, 20.6, and 20.4 ng/mL for CON, MEL, and TRANS, respectively; SEM = 1.0). The impact of long-distance transportation on cortisol has been variable, with research studies reporting increased or unaltered circulating cortisol concentrations following transport (Swanson and Morrow-Tesch, 2001). However, previous research from our group reported increased plasma cortisol concentrations during feedlot receiving in cattle exposed to the same transportation schedule adopted herein (Marques et al., 2012; Cooke et al., 2013a; Cooke et al., 2013b). Hence, the lack of treatment effects on plasma cortisol in the present experiment, particularly between CON and TRANS steers, was unexpected and may have hindered proper assessment of meloxicam effects on transport-induced plasma cortisol Table 1. Feedlot receiving performance (21 d) of steers transported for 1,440 km and receiving meloxicam (MEL; 1 mg/kg of BW daily; n = 7) or lactose monohydrate (TRANS; 1 mg/kg of BW daily; n = 7) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving, or non-transported steers that concurrently received lactose monohydrate (CON; 1 mg/kg of BW daily; n = 7).

Item	CON	MEL	TRANS	SEM	P=
BW, kg					
Initial	259.9	260.4	260.3	4.9	0.99
Final	291.5	292.9	287.7	5.5	0.78
Shrink, %	-0.71 ^a	9.07 ^b	9.83 ^b	0.35	< 0.01
ADG, kg/d	1.50 ^a	1.48 ^a	1.26 ^b	0.07	0.03
DMI, kg/d					
Hay	5.90	5.98	5.75	0.09	0.19
Concentrate	2.39	2.38	2.38	0.01	0.18
Total	8.33	8.34	8.10	0.09	0.13
G:F, g/kg	185 ^a	177 ^a	153 ^b	8	0.03

¹Within rows, values with different superscripts differ ($P \le 0.05$).

response. Nevertheless, Van Engen et al. (2014) also did not detect significant differences in plasma cortisol concentrations during feedlot receiving between steers transported for 16 h and orally administered meloxicam or a whey protein placebo prior to transport.

A treatment × day interaction was detected for serum NEFA (P < 0.01; Figure 1), given that NEFA concentrations were greater (P < 0.01) for TRANS and MEL compared with CON steers on d 1 of feedlot receiving. These results corroborate that stress due to long-distance transport stimulates fat tissue mobilization and increases circulating NEFA concentration in cattle (Marques et al., 2012), whereas oral meloxicam administration did not alleviate this outcome. Newby et al. (2013) also administered meloxicam (0.5 mg/kg of BW) subcutaneously to Holstein cows approximately 24 h after parturition, and reported that serum NEFA concentrations during the initial 12 d of lactation were similar compared with cohorts receiving saline. Hence, meloxicam administration appears not to modulate lipid mobilization in cattle upon stress and nutritional challenges.

A treatment × day interaction was detected for plasma haptoglobin (P < 0.01; Figure 1), whereas a tendency (P =0.09; Figure 1) for the same interaction was detected for plasma ceruloplasmin. Corroborating the ADG, DMI, G:F, and physiological differences detected between TRANS and CON steers, previous research from our group also reported that 24-h road transport elicited an acute-phase response that reduced feedlot receiving performance of feeder cattle (Cooke et al., 2012; Marques et al., 2012; Cooke et al., 2013b). Accordingly, circulating concentrations of acutephase proteins in transported cattle have been negatively associated with receiving ADG (Qiu et al., 2007; Araujo et al., 2010), and such outcome can be attributed to altered basal metabolism, increased tissue catabolism, and reduced feed intake and efficiency during an acute-phase response (Johnson, 1997). The reason why plasma haptoglobin



Figure 1. Concentration of serum NEFA (1A), plasma haptoglobin (1B) and ceruloplasmin (1C) in steers transported for 1,440 km and receiving meloxicam (**MEL**; 1 mg/kg of BW daily; n = 7) or lactose monohydrate (**TRANS**; 1 mg/kg of BW daily; n = 7) at loading (d 0), unloading (d 1), and daily from d 2 to 7 of feedlot receiving, or non-transported steers that concurrently received lactose monohydrate (**CON**; 1 mg/kg of BW daily; n = 7). Within days, letters indicate treatment differences ($P \le 0.05$); a = TRANS vs. CON, b = MEL vs. CON, c = TRANS vs. MEL.

concentrations increased on d 10 of feedlot receiving in CON, but not MEL and TRANS steers, is unknown. A similar response was not detected for plasma ceruloplasmin, whereas circulating concentrations of acute-phase proteins are typically correlated (Cooke et al., 2009; Araujo et al., 2010). Haptoglobin is also positively associated with morbidity in cattle (Petersen et al., 2004), but no incidence of morbidity or mortality was detected during feedlot receiving. In addition, hay, concentrate, and total DMI of CON were similar ($P \ge$ 0.42) compared with MEL and TRANS steers during the second week of feedlot receiving, whereas an inflammatoryinduced haptoglobin response is usually accompanied by reduced feed intake (Johnson, 1997; Araujo et al., 2010).

Supporting our hypothesis, meloxicam administration alleviated the acute-phase response elicited by 24-h transport based on differences detected for plasma haptoglobin and ceruloplasmin between TRANS and MEL steers. Meloxicam inhibits cyclooxygenase, an enzyme that regulates synthesis of inflammatory eicosanoids associated with the acute-phase response such as PGE2 (Lees et al., 2004). Accordingly, Van Engen et al. (2014) orally administered meloxicam or a whey protein placebo at approximately 1 mg/kg of BW to steers prior to a 16-h road transport. These authors reported that steers receiving meloxicam had reduced circulating concentrations of biomarkers of stress and inflammation compared with cohorts receiving placebo, including stressinduced neutrophilia, as well as monocyte and lymphocyte counts. However, Van Engen et al. (2014) did not evaluate production parameters to determine if the immunological benefits of oral meloxicam administration to transported cattle would result in enhanced feedlot receiving performance. In the present experiment, feedlot receiving performance of MEL steers was similar compared with CON and greater compared with TRANS. These results indicate that oral meloxicam administration effectively prevented the performance losses caused by road transport, likely by inhibiting the changes in metabolism and feed intake regulated by inflammatory eicosanoids during an acute-phase response (Johnson, 1997; Klasing and Korver, 1997; Lees et al., 2004).

IMPLICATIONS

Meloxicam administration to feeder steers prior to road transport, at feedlot arrival, and during the initial week of feedlot receiving (1 mg/kg of BW/administration) reduced the acute-phase protein response and increased ADG, DMI, and G:F during feedlot receiving. Hence, meloxicam administration may be a viable strategy to mitigate inflammatory reactions and performance losses elicited by long-distance transportation.

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Effects of lasalocid and diet particle size on feedlot performance, carcass traits, and nutrient digestibility in feedlot lambs

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ABSTRACT: The objective of this research was to determine the influence of lasalocid (Bovatec, Alpharma Inc.) and diet particle size on feed offered, nutrient digestibility, N balance, growth performance, and carcass characteristics in lambs consuming a feedlot diet. One hundred sixty cross bred (Suffolk x Rambouillet) lambs $(31.2 \pm 0.09 \text{ kg})$ were utilized in a completely random design and placed into 16 feedlot pens (4 pens/treatment; 10 lambs/pen) for a 112 d finishing study. Lambs were fed a basal feedlot diet of 80% corn and 20% market lamb pellet (DM basis) ad libitum. Treatments were whole corn with lasalocid (WCL), whole corn without lasalocid (WCNL), ground corn with lasalocid (GCL), or ground corn without lasalocid (GCNL). Lasalocid was provided through the market lamb pellet. Two-day weights were taken at the beginning and end of the trial, and single day weights taken on d 33, 57, and 85. One hundred fifty five lambs (69.7 \pm 0.74 kg BW) were harvested on d 116 at a commercial abattoir. Carcass data were collected 24 h after chill. There were no differences among treatments for final BW, feed offered, G:F, mortality, HCW, leg score, conformation score, fat depth, body wall thickness, flank streaking, quality or yield grade and dressing percentage ($P \ge$ 0.06) in the feedlot study. However, there was an interaction of particle size and use of ionophores for ADG (P = 0.05), Loin eye area (P < 0.001), and % boneless closely trimmed retail cuts (%BCTRC; P = 0.004). Loin eye area was greatest (P < 0.05) for WCL and GCNL, with GCL intermediate. However, GCNL had the greatest (P < 0.05) %BCTRC. A second study was conducted utilizing the same treatments to evaluate N balance in 16 crossbred wethers (Suffolk x Rambouillet; 40 ± 1.7 kg BW). N balance was not affected by treatment (P = 0.22). These results indicate that a ground corn diet without lasalocid can increase %BCTRC and loin eye area without affecting N balance.

Key words: sheep, ionophores, lasalocid, particle size, performance

INTRODUCTION

Typically, lambs are fed whole corn accompanied by a market lamb pellet through self-feeders during the growing-finishing phase. However, as evidenced in the cattle industry, when feeding high energy and low roughage diets, acidosis can become a health problem (Elam, 1976).

Research in cattle has shown the effectiveness of ionophores for increasing feed efficiency and decreasing the incidence of acidosis (Jacques et al., 1987) in high grain diets. Additionally, research has been conducted reporting

their effectiveness in sheep (Funk et al., 1986; Horton, 1980; Ricke et al., 1984). However, monensin is not currently labeled for use in sheep (FDA, 2005). The ionophore lasalocid is approved for use in sheep (FDA, 2003) and has been shown to increase total tract organic matter digestibility in lambs (80 vs. 76.4%; Funk et al., 1986). It has also been reported that grinding the diet can increase digestibility, intake, and performance of livestock, however, in the lamb finishing industry producers typically do not grind or crack grains (Kerley, et al., 1985). One potential reason is that when feeds are ground, lambs tend to sort to select larger particles (Reynolds and Lindahl, 1960), resulting in reduced DM intake. Grinding feeds can also increase the rate of digestion, therefore decreasing total digestibility (Reynolds and Lindahl, 1960), tending to result in more cases of acidosis (Gressley, et al., 2011).

There is limited research in the area of particle size, especially when coupled with ionophores and their effect on nutrient digestibility, in sheep. The hypothesis for our research was that lambs fed ground rations and lasalocid would have the greatest performance in the feedlot and improved nutrient digestibility when compared to lambs fed rations that weren't ground or rations without lasalocid. The objectives used to test this hypothesis were to determine the influence of lasalocid and particle size of the diet on feed intake, growth performance, and carcass characteristics as well as N balance in lambs consuming a feedlot diet.

MATERIALS AND METHODS

All procedures were approved by the Animal Care and Use Committee of North Dakota State University (protocol # A13041). This study was conducted at the NDSU Hettinger Research Extension Center in Hettinger, ND.

Feedlot Study

Animals and Diets. At 2 wk of age, tails were docked, males were castrated, and all lambs were vaccinated (**CD**¬**T**; 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 pellet diet following weaning. Lambs were vaccinated with CD-T again at 60 d of age (weaning) and d -1 of the study. In May of 2013, 160 crossbred (Suffolk x Rambouillet) lambs were stratified by BW $(31.2 \pm 0.09 \text{ kg}; \text{ approximate } 90 \text{ kg}; \text{ appr$ d of age) and sex (80 wethers and 80 ewes) and randomly assigned to 1 of 16 outdoor pens (10 lambs/pen). Pens were assigned randomly to 1 of 4 treatments, with pen serving as the experimental unit (n = 4 pens/treatment). Treatments were: whole corn with lasalocid (WCL), whole corn without lasalocid (WCNL), ground corn with lasalocid (GCL), or ground corn without lasalocid (GCNL; Table 1). Lambs receiving lasalocid (Bovatec, Alpharma Inc., Bridgewater, NJ) received the basal feedlot ration with lasalocid included in the market lamb pellet starting on d 0. A factorial arrangement of treatments was applied in a completely randomized design to evaluate the outlined objectives.

Ground diets were ground through a 1.27 cm screen (Gehl Mix-All, Model 170, Gehl, West Bend, WI). Diets were mixed and provided by the same mixer-grinder and offered ad libitum via bulk feeders (48.6-cm bunk space/lamb). Lambs had continuous access to clean, fresh water and shade. Study diets were balanced to be equal to or great than CP and energy (NE) requirements (NRC, 2007). The rations were formulated to have a minimum Ca:P ratio of 2:1. Feeders were checked daily and cleaned of contaminated feed. Lambs were observed daily to monitor health and treated when necessary.

Data Collection Procedures. The study was divided into four periods. Lambs were weighed on 2 consecutive d at the initiation (d -1 and 0) and end (d 111 and 112) of the trial; single day weights were taken on d 33, 57, and 85 and used to assist in evaluation of morbidity. Ration and feed ingredient samples from the bulk feeders were taken 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 using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2-mm screen. Samples were analyzed for DM, ash (method 942.05; AOAC Int., 2010), N (method 2001.11; AOAC Int., 2010) using a Kjeltec Auto 1030 Analyzer (Tecato AB, Höganäs, Sweden), 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). After 2 d BW on d 112, all lambs (115 hd; 69.7 ± 0.74 kg BW) were harvested at Superior Farms Inc. (Denver, CO).

Trained personnel collected carcass data after a 24-h chill (temperature $< 2^{\circ}$ C and humidity near 100%). Carcass data collected included HCW, leg score, conformation score, fat depth (over the 12th rib), body wall thickness, loin eye area, flank streaking, quality grade, and yield grade, and % boneless closely trimmed retail cuts (%BCTRC; Savell and Smith, 2000).

Statistical Analyses. All data from the feedlot study was analyzed as a completely randomized design with a 2 • 2 factorial arrangement of treatments using the MIXED procedures (SAS Inst. Inc., Cary, NC) with pen serving as the experimental unit. The model included effects of inclusion or exclusion of ionophores and the diet particle size (ground or whole), and the interaction of both the ionophores and diet processing with P-value ≤ 0.05 considered significant. LS Means were utilized to partition treatment differences (P < 0.05).

Nitrogen Balance Study

Animals and Treatments. Sixteen Suffolk x Rambouillet wethers (40 ± 1.7 kg BW; approximate age = 90 d) were used in completely random design. Wethers were weighed on d 0 and 1, stratified by weight, and allotted randomly to treatments (n = 4 wethers/treatment) as described in the feedlot trial. 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

	Treatments ¹						
Item	WCNL	WCL	GCNL	GCL			
Ingredient, %							
Corn	80	80	80	80			
Market lamb pellet ²	20	20	20	20			
Nutrient composition, %							
DM	96.4	96.3	96.0	96.7			
CP	19.6	18.7	17.7	17.2			
NDF	16.4	15.4	14.4	15.7			
ADF	5.3	4.8	4.5	5.3			

Table 1. Ingredient and nutritional composition of diets fed to lambs fed differing particle sizes of corn and market lamb pellets with or without lasalocid (DM basis)

¹Treatments: WCNL= whole corn and market lamb pellet without lasalocid (Bovatec, Alpharma Inc., Bridgewater, NJ); WCL= whole corn and market lamb pellet with lasalocid; GCL= ground corn and market lamb pellet without lasalocid; GCL= ground corn and market lamb pellet with lasalocid.

²Market lamb pellet contained 38% CP, 4.25% Ca, 0.6% P, 3.5% salt, 1.2 mg/kg Se, 52,920 IU/kg vitamin A, 5,292 IU/kg vitamin D, and 209 IU/kg vitamin E with treatments WCNL and GCNL having no lasalocid and WCL and GCL containing 20 g/ton of lasalocid.

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 21 d. Dry matter intake was determined on d 14 to 20. Additionally, samples of corn, pellets, and ration were collected on d 14 to 20 and dried at 55°C for 48 h. Wethers were fitted with fecal collection bags on d 11. Total fecal and urine output were collected on d 15 to 21. A subsample of each daily fecal sample (7.5% of total, wet basis) was dried at 55°C for 96 h for calculation of fecal DM. Urine was 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 vaccutainers (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 through a Wiley mill (2-mm screen) and composited by lamb. Daily samples of corn, pellets 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 in the feedlot study. Feed, orts, fecal, and urine samples were analyzed for N as described previously in the feedlot study. Concentration of N in feed, orts, fecal, and urine samples was used to calculate daily N 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). 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).

Statistical Analyses. Lamb N balance data were analyzed as a randomized design using the MIXED procedure of SAS with animal serving as experimental unit. Repeated measures were used to analyze day effect for serum urea-N. The model included treatment, day, and treatment • day interaction. The covariance structure used was Autoregressive (1). Other structures were tested but Autoregressive (1) was the best fit. Significance was observed at $P \le 0.05$. LS Means were utilized to partition treatment differences (P < 0.05).

RESULTS AND DISCUSSION

Feedlot Study

Results for feedlot lamb growth, carcass characteristics and mortality are reported in Table 2. There were no interactions among treatments for final BW, feed offered, G:F, mortality, HCW, leg score, conformation score, fat depth, body wall thickness, flank streaking, quality or yield grade, and dressing percentage ($P \ge 0.06$). However, there was an interaction of particle size and use of ionophores for ADG (P = 0.05), loin eye area (P < 0.001), and BCTRC (P = 0.004). While an interaction was observed for ADG, upon comparison of means no differences were observed among treatments (P > 0.05). Numerically, the WCL fed lambs had the highest ADG, and this difference in the gains could be relevant from a producer standpoint with a difference of 2.4 kg over the 120 d finishing period when comparing WCL vs. WCNL or GCL. In a study by Erickson et al., (1989), whole vs. ground corn diets had no affect (P > 0.05) on ADG or G:F in feedlot lambs. A tendency (P = 0.06) was observed for an interaction of particle size and ionophore for final BW, where WCL fed lambs tended to be heavier than the other lambs by up to 0.15 kg. Similarly, Erickson et al. (1988), reported a tendency for lambs fed whole grain diets to have heavier final BW.

Loin eye area was greatest (P < 0.05) for WCL and GCNL, with GCL intermediate. However, GCNL had the greatest (P < 0.05) %BCTRC. Additionally, lambs fed lasalocid had heavier (P = 0.05) HCW compared to those fed diets without lasalocid. In a previous trial at this lab, lambs which were fed coarse rolled corn with lasalocid had higher dressing percentage when compared to lambs not receiving lasalocid, however lasalocid did not affect feedlot performance or any other carcass characteristics (Rupprecht et al., 1992). Similarly, Erickson et al. (1988) reported that lambs fed whole vs. ground corn had no difference in HCW and leg score, however lambs had higher yield grades and thicker fat depths when fed whole grains vs. ground (Erickson et al., 1988). Reynolds and Lindahl (1960) reported that lambs tend to not consume finely ground feeds and sort through feed to select larger particles and that secondly, grinding increases the rate of digestion, therefore decreasing total digestibility.

Limited research is available on the effects of lasalocid or particle size on carcass characteristics in lambs; therefore it is difficult to discuss these results in relation to previous research. In a study by Paterson et al., (1983), rumen propionate was increased and the acetate to propionate ratio was decreased when lasalocid was fed to lambs. The addition of lasalocid to a low ruminal N degradable feed resulted in more rapid weight gain than without lasalocid; however, when lambs were fed soybean meal with or without lasalocid, lasalocid actually slowed the rate of gain (Paterson et al., 1983). These results indicates that lasalocid is effectively increasing propionate production in the rumen, which explains the tendency for higher growth performance of the lambs fed WCL diets in the current trial. However, GCNL fed lambs had similar growth performance to WCL fed lambs, which is quite interesting. In the current trial, GCNL fed lambs did have greater %BCTRC, however these lambs also had increased loin eve area, although statistically similar to WCL, which could have driven the increase in %BCTRC. Interestingly, the current trial showed that particle size also affected loin eye area (P = 0.008), while prior research in ground vs. whole grains has shown no effect of diet processing on loin eye area (Erickson et al., 1988; Erickson et al., 1989).

Table 2. Effects of lasalocid and particle size on lamb growth performance, carcass characteristics, and mortality

	Treatment						P-value3	
Item	WCNL	WCL	GCNL	GCL	SEM ²	PS	ION	PS*ION
Initial BW, kg	31.2	31.3	30.8	31.3	0.1			
Final BW, kg	68.7	71.4	69.6	69.1	0.7	0.34	0.17	0.06
ADG, kg/d	0.26 ^a	0.28 ^a	0.27 ^a	0.26 ^a	0.01	0.76	0.73	0.05
Feed offered, kg DM • head ⁻¹ • d ⁻¹	2.52	2.67	2.60	2.67	0.067	0.58	0.14	0.57
G:F	0.10	0.10	0.11	0.10	0.004	0.59	0.47	0.23
Mortality, %	0	5	2.5	5	2.39	0.61	0.14	0.61
HCW, kg	33.7	35.4	34.0	34.3	0.48	0.37	0.05	0.16
Leg score ⁴	11.5	11.7	11.5	11.6	0.13	0.06	0.14	0.94
Conformation score ⁴	11.7	11.9	11.8	11.8	0.10	0.85	0.24	0.51
Fat depth, cm ⁵	0.73	0.61	0.66	0.74	0.06	0.60	0.72	0.11
Body wall thickness, cm	2.53	2.55	2.51	2.63	0.08	0.73	0.42	0.54
Loin eye area, cm ²	19.6 ^a	21.1 ^{bc}	22.0 ^c	20.7 ^b	0.31	0.008	0.79	< 0.001
Flank streaking ⁶	375	389	366	376	9	0.27	0.22	0.89
Quality grade ⁴	11.7	11.8	11.6	11.6	0.09	0.10	0.43	0.88
Yield grade ⁷	3.28	2.81	3.01	3.30	0.22	0.61	0.70	0.11
BCTRC, % ⁸	46 ^a	47 ^a	47 ^b	46 ^a	0.18	0.06	0.24	0.004
Dressing, %	49	50	49	50	0.39	0.80	0.10	0.85

¹Treatments: WCNL= whole corn and market lamb pellet without lasalocid (Bovatec, Alpharma Inc., Bridgewater, NJ); WCL= whole corn and market lamb pellet without lasalocid; GCL= ground corn and market lamb pellet without lasalocid; GCL= ground corn and market lamb pellet with lasalocid.

 $^{2}n = 4.$

³PS= particle size of diet and ION= ionophores.

⁴Leg score, conformation score and quality grade: 1= cull to 15= high prime.

5Fat depth and yield grades.

⁶Flank streaking: 100 to 199= practically devoid; 200 to 299= traces; 300 to 399= slight; 400 to 499= small; 500 to 599= modest.

⁷Yield grade= 0.4 + (10 × fat depth).

⁸ BCTRC= boneless closely trimmed retail cuts, $\% = [49.936 - (0.0848 \times 2.205 \times HCW, kg) - (4.376 \times 0.3937 \times fat depth, cm) - (3.53 \times 2.205 \times HCW, kg)$

 $0.3937 \times \text{body wall thickness, cm} + (2.456 \times 0.155 \times \text{loin eye area, cm}^2)].$

^{a,b,c}Means within a row with different superscripts differ (P < 0.05).

Table 3	. Effects of lasalocid	and particle s	size of feed or	N intake	, excretion,	balance,	and serum	urea-N	concentratio	n in
lambs		-								

	Treatment						P-value	3
Item	WCNL	WCL	GCNL	GCL	SEM ²	PS	ION	PS*ION
Daily DMI, g/ kg BW	38.43	36.99	66.16	37.97	12.9	0.29	0.28	0.32
Daily N intake, g/ kg BW	1.06	0.94	1.11	1.04	0.06	0.26	0.18	0.69
Daily N excretion, g/ kg BW								
Fecal	0.23	0.14	0.21	0.16	0.01	0.87	0.01	0.45
Urinary	0.05	0.04	0.05	0.05	0.004	0.23	0.97	0.25
Daily N Balance, g/ kg BW	0.78	0.76	0.84	0.83	0.05	0.22	0.76	0.99
Serum urea-N ⁴ , mM	10.29	10.11	10.87	10.37	0.53	0.50	0.65	0.77

¹Treatments: WCNL= whole corn and market lamb pellet without lasalocid (Bovatec, Alpharma Inc., Bridgewater, NJ); WCL= whole corn and market lamb pellet with lasalocid; GCNL= ground corn and market lamb pellet without lasalocid; GCL= ground corn and market lamb pellet with lasalocid.

 $^{2}n = 4.$

³PS= particle size of the diet and ION= ionophores.

⁴P-values for serum urea-N: day (P = 0.0018) and treatment × day (P = 0.33).

Nitrogen Balance Study

There were no interactions among treatments for DMI, N intake, N balance, or serum urea-N concentration (Table 3; $P \ge 0.18$), however, there was a day effect (P = 0.0018) for serum urea-N concentration. Days 1, 2, and 3 were generally lower than days 4 to 6 (P < 0.05). However, the addition of lasalocid did decrease (P = 0.01) fecal N excretion. This is similar to findings by Ricke et al., (1984), in which lasalocid

treated lambs also had decreased fecal N excretion when compared to lambs fed monensin or no ionophore. Varying results exist on lasalocid's effects on N digestibility, with some reporting it increases N digestibility (Paterson et al., 1983; Ricke et al., 1984), while other report that it remains unaffected (Funk et al., 1986) with N balance also appearing to remain unaffected (Funk et al., 1986). Ricke et al., (1984) also reports that lasalocid-fed lambs had less fecal N loss and therefore higher N retentions, which could be reflective of increased digestibility. The differences in findings could be due to the different types of collection, ranging from N balance trials, to in situ techniques.

There is conflicting research on particle size and its effects on N digestion, N balance, and serum urea-N concentration. Although there was no particle size affect ($P \ge 0.22$) in the current trial, previous research by Kerley et al., (1985) reported that N digestion was increased in lambs fed 6.5, 5.4 and 0.8 mm particle size corncob diets, while the 1.4 mm diet was decreased. The 1.4 mm diet also had higher fecal N loss when compared to the other diets. Other research by Perez-Torres et al. (2011) reports no differences in DM or OM intake or digestibility in diets that differ in particle size, agreeing with results from the current trial.

IMPLICATIONS

We reject our initial hypotheses that unground rations including lasalocid would have improved performance compared to ground rations and rations without lasalocid. In our trials, whole grain diets that include lasalocid may increase average daily gain and final body weight, but the results are inconclusive at this time. Additionally, our results did not support previous researchers in their conclusion that ground diets increased passage rate, and subsequently mortality due to acidosis. Lasalocid also may increase hot carcass weight in both ground and whole diets, which would be beneficial for lamb producers getting paid for hot carcass weight, vs. live weight, but did not appear to play a role in lamb mortality.

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Metabolic Imprinting: impact on growth and reproductive development of beef heifers

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INTRODUCTION

ABSTRACT: This experiment compared development of beef heifers receiving metabolic imprinting (MI) or not (CON) while nursing. Sixty Angus × Hereford heifers (initial age 68 ± 3 d; initial BW 140 ± 3 kg) were utilized. On d 0, heifers were ranked by initial BW, and assigned to pairs in a manner that heifers within each pair had similar BW. Pairs were randomly assigned MI or CON. From d 0 to 51, MI pairs and their respective dams were allocated to 15 drylot pens and had a creep-feeder that allowed heifers to have ad libitum access to a corn-based supplement. The CON heifers and their dams were maintained in an adjacent drylot pen. From d 52 to 111, cows and heifers from both treatments were managed as a single group on a semiarid range pasture. On d 111, heifers were weaned and allocated to 2 pastures according to treatment until d 277, receiving 4.8 kg/heifer daily of alfalfa-grass hay in addition to 2.5 kg/heifer daily of a corn-based concentrate. Full BW was recorded prior to (d -1 and 0) and at the end of imprinting (d 50 and 51), whereas shrunk BW was collected on d 118, 190, and 277. On d 0, 50, 117, 189, and 258, heifers were evaluated for LM depth and backfat thickness via ultrasonography. Blood samples were collected on d 0, 51, 113, 187, and 261 to determine plasma insulin, glucose, and IGF-1 concentrations, as well as every 10 d beginning on d 113 to assess heifer puberty attainment via plasma progesterone. From d 0 to 51, MI heifers had greater (P < 0.01) ADG compared with CON cohorts, whereas ADG did not differ among treatments during the subsequent evaluations ($P \ge 0.20$). On d 51, MI heifers had greater (P < 0.01) plasma glucose and IGF-I concentrations compared with CON, whereas plasma insulin concentration was greater (P < 0.01) for CON compared with MI heifers on d 261. No treatment effects were detected ($P \ge$ 0.28) for backfat thickness and LM depth. Heifers receiving MI attained puberty earlier (P = 0.02) during the experiment compared with CON heifers, although no treatment effects were detected for heifer age and BW ($P \ge 0.52$) at puberty. Hence, providing a corn-based supplement ad libitum through a creep-feeder for 50 d to nursing heifers did not hasten their fat accretion or puberty attainment later in life.

Keywords: Beef heifers, metabolic imprinting, performance, puberty

For optimal economic return and lifetime productivity, replacement beef heifers should attain puberty by 12 months of age (Lesmeister et al., 1973). Age at puberty in cattle is greatly influenced by nutritional status and body development (Schillo et al., 1992), including rate of body fat deposition and subsequent circulating concentrations of leptin (Garcia et al., 2002). Accordingly, nutritional alternatives that enhance carcass lipogenesis may hasten puberty attainment in heifers (Williams et al., 2002).

Metabolic imprinting, which is an epigenetic response to a nutritional challenge during early life that permanently alters physiological outcomes in later life (Lucas, 1991; Du et al., 2010), has been shown to promote the physiological mechanisms associated with fat accretion in cattle (Graugnard et al., 2010). McCann et al. (2011) reported that feeding a highenergy supplement to early-weaned beef calves from 100 to 205 days of age enhanced carcass marbling compared with calves weaned at 205 d. Hence, metabolic imprinting may be an alternative to hasten puberty attainment in growing heifers by increasing rate of fat accretion, although this hypothesis has not been tested to date. In addition, research studies evaluating the metabolic imprint concept in beef cattle have utilized early-weaned calves, which prevented proper separation of weaning and nutritional effects (Scheffler et al., 2014), whereas early-weaning may not be a feasible management alternative for many commercial cow-calf systems. One alternative to isolate the metabolic imprinting concept from early weaning is to provide supplements to nursing heifers via creep-feeding. Hence, this experiment compared growth, body composition, physiological parameters, and puberty attainment of nursing beef heifers receiving or not metabolic imprinting via ad libitum access to creep-feeding.

MATERIAL AND METHODS

Animals and diets. Sixty nulliparous Angus × Hereford heifers (initial age 68 ± 3 d; initial BW 140 ± 3 kg) were utilized in this experiment, which was divided into three phases: imprinting phase (d 1 to d 51), pre-weaning phase (d 52 to d 111), and development phase (d 112 to d 277). On d 0, heifers were ranked by initial BW, and assigned to pairs in a manner that both heifers within each pair had similar initial BW. On d 0, pairs were randomly assigned to: 1) metabolic imprinting (**MI**), or 2) control (**CON**).

During the imprinting phase, MI heifers and their respective dams were allocated to 15 drylot pens (2 cow-calf pairs/pen). Each pen had a creep-feeder that allowed the heifers to have ad libitum access to a grain-based supplement (70% corn, 15% soybean meal, 10% dehydrated alfalfa, and 5% molasses; as-fed basis). The CON heifers and their respective dams were maintained in an adjacent single drylot pen (50 × 100 m). Cows from both treatments received 8.1 kg/cow daily of meadow-grass hay (56% TDN, 65% NDF, 41% ADF, 1.04 Mcal/kg of NE₁, 1.07 Mcal/kg of NE_m, 0.48 Mcal/kg of NE_g, and 8.2% CP) and 5.4 kg/cow daily of mixed alfalfa-grass hay (61% TDN, 42% NDF, 34% ADF, 1.38 Mcal/kg of NEI, 1.30 Mcal/kg of NE_m, 0.73 Mcal/kg of NE_g, and 19% CP).

During the pre-weaning phase, cows and heifers from both treatments were managed as a single group on a 6,500 ha semiarid range pasture (Ganskopp and Bohnert, 2009). On d 111, heifers were weaned and allocated to 2 meadow foxtail (*Alopecurus pratensis L.;* 6 ha each) pastures harvested for hay the previous summer according to treatment for the development phase. Heifers were rotated between pastures every 10 d, and received 4.8 kg/heifer daily (DM basis) of the same mixed alfalfa-grass hay offered during the imprinting period, in addition to 2.5 kg/daily of a corn-based concentrate (72% corn and 28% soybean meal; as-fed basis).

Sampling. For ADG calculation, heifers were weighted on 2 consecutive days to determine full BW prior to (d -1 and 0) and at the end of the imprinting phase (d 50 and 51), whereas individual shrunk BW (after 16h of feed and water restriction) was collected on d 118, 190, and 277 of the development phase.

On d 0, 50, 117, 189, and 258, heifers were evaluated for LM depth and backfat thickness via real-time ultrasonography. Ultrasound measurements were obtained at the 12th to 13th rib interface using an Aloka 500V (Aloka Co., Ltd., Wallingford, CT) B-mode instrument equipped with a 3.5-MHz, 125 mm general-purpose transducer array (UST-5011U-3.5). A single technician collected images with software from the Cattle Performance Enhancement Company (CPEC, Oakley, KS). Estimates of LM depth and backfat thickness were based on image analysis programming (Brethour, 1994) within the CPEC software.

Blood samples were collected on d 0, 51, 113, 187, and 261 to determine plasma insulin, glucose and IGF-1 concentrations. During the development phase, blood samples were also collected on 10-d intervals to assess heifer puberty attainment via plasma progesterone. Heifers were considered pubertal once plasma progesterone concentrations were \geq 1.0 ng/mL for 2 consecutive weeks (Perry et al., 1991), and puberty attainment was declared at the first week of increased progesterone. Age and BW at puberty were determined according to the week of puberty attainment, heifer shrunk BW recorded in the weighing immediately prior to puberty attainment, and subsequent heifer ADG.

Blood samples were collected via jugular venipuncture into commercial blood collection tubes containing 158 USP units of freeze-dried sodium heparin for plasma collection. All blood samples were placed immediately on ice, subsequently centrifuged (2,500 \times g for 30 min; 4°C) for plasma harvest, and stored at -80°C on the same day of collection. Plasma insulin and progesterone were analyzed using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Plasma glucose was determined using a quantitative colorimetric kit (#G7521; Pointe Scientific, Inc., Canton, MI). Concentrations of IGF-I were determined using a humanspecific commercial ELISA kit (SG100; R&D Systems, Inc., Minneapolis, MN) with 100 % cross-reactivity with bovine IGF-I, previously validated for bovine samples (Cooke et al., 2012).

Statistical analysis. Growth, body composition, and physiological data were analyzed using the MIXED procedure (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for ADG, age at puberty, and BW at puberty contained the fixed effect of treatment. The model statement used for analysis of blood variables and body composition contained the fixed effects of treatment, day, and the resultant interaction. Body composition and blood variables were adjusted covariately to values obtained on d 0. Data were analyzed using pair(treatment) and heifer(pair) as random variables. The specified term used in the repeated statement for body composition and blood variables was day, the subject was heifer(pair), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Results are reported as least square means, or covariately adjusted means for body composition and blood variables, and separated using LSD. Puberty attainment was analyzed using survival analysis (LIFETEST procedure of SAS) by regressing the proportion of pubertal heifers on day of the experiment. Differences between treatment survival curves were determined by the Wilcoxon test. Significance was set at $P \le 0.05$, and tendencies were determined if P > 0.05 and $P \le 0.10$. Results are reported according to treatment effects if no interactions were significant or according to the highestorder interaction detected.

RESULTS AND DISCUSSION

During the imprinting phase, average intake of the cornbased concentrate by MI heifers was $1,025 \pm 128$ g/heifer daily. As expected, MI heifers had greater (P < 0.01) ADG and during the imprinting phase, which resulted in a tendency (P=0.10) for a greater BW at the end of the imprinting period compared with CON cohorts (Table 1). Based on the average BW during the imprinting period, intake of the corn-based concentrate by MI heifers was $0.83\% \pm 0.09$ of heifer BW. During the pre-weaning phase, ADG was similar (P = 0.80) between treatments, whereas weaning BW still tended (P =

Table 1. Body weight and ADG of beef heifers receiving a combased supplement ad libitum through a creep-feeder for 50 d while nursing their damns (**MI**; n = 15), or cohorts that did not receive the supplement (**CON**; n = 15).¹

Item	CON	MI	SEM	P-Value						
Imprinting phase (d 0 to	Imprinting phase (d 0 to 51)									
BW d 0, kg	103	105	6	0.84						
BW d 51, kg	127	143	6	0.10						
ADG, kg/d	0.49	0.75	0.03	< 0.01						
Pre-weaping phase (d 52 to 111)										
BW d 118, kg	161	175	6	0.10						
ADG, kg/d	0.50	0.49	0.03	0.80						
Development phase (d 111 to 277)										
BW d 277, kg	292	299	9	0.63						
ADG, kg/d	0.82	0.78	0.02	0.20						

¹ Values reported on d 0 and 51 are the average of full BW collected on d -1 and 0, and 50 and 51, respectively. On d 118 and 277, shrunk BW was recorded after a 16 h of feed and water restriction. Average daily gain was calculated based on the following recorded BW: Imprinting phase, d 0 and 51; pre-weaning phase, d 51 and 118, development phase, d 118 and 277.

0.10) to be greater for MI compared with CON heifers (Table 1). During the development phase, ADG remained similar (P = 0.20) between CON and MI heifers, although the BW differences disappeared at the end of the development phase (P = 0.63). Hence, the metabolic imprinting process adopted herein did not result in hastened growth when heifers from both treatments were managed similarly prior to and after weaning.

Treatment × day interactions were detected for plasma concentrations of glucose, insulin, and IGF-I (P < 0.05; Table 2). On d 51, MI heifers had greater plasma glucose and IGF-I concentrations compared with CON heifers, which can be directly attributed to the concentrate intake during the imprinting phase (Hess et al., 2005). The similar plasma IGF-I and glucose concentrations between treatments on d 187 and 261 also corroborate with the similar ADG and management of MI and CON heifers (Wettemann and Bossis, 2000). Conversely, plasma insulin concentrations were similar on d 51, but greater for CON compared with MI heifers on d 261, despite similar ADG and dietary management between treatments (Hess et al., 2005).

No treatment effects were detected for backfat thickness (P = 0.43) and LM depth (P = 0.28). Heifers receiving MI attained puberty earlier (P = 0.02) during the experiment compared with CON heifers (Figure 1). However, no treatment effects were detected for heifer age (P = 0.52) and BW (P = 89) at puberty attainment, suggesting that treatment effects detected for puberty attainment should be mainly attributed to greater BW of MI heifers at weaning. These results do not support our hypothesis, given that the metabolic imprinting process adopted herein did not impact fat accretion, plasma hormones that regulate puberty attainment such as IGF-I (Jones et al., 1991) during the peripubertal period, nor stimulated heifers to reach puberty at lighter BW or younger age.

Table 2. Body composition, puberty attainment, and plasma concentrations of glucose, insulin, and IGF-I in beef heifers receiving a corn-based supplement ad libitum through a creep-feeder for 50 d while nursing their damns (**MI**; n = 15), or cohorts that did not receive the supplement (**CON**; n = 15).¹

Item	CON	MI	SEM	P-Value
Body composition				
Backfat thickness, mm	3.89	4.00	0.09	0.43
LM depth, mm	46.5	47.6	0.7	0.28
Puberty attainment				
Age at puberty, d	307	300	7	0.52
BW at puberty, kg	262	263	7	0.94
Plasma glucose, mg/dL				
d 51	69.1	75.9	1.6	< 0.01
d 113	66.2	67.	1.6	0.63
d 187	73.7	71.0	1.6	0.26
d 261	75.1	78.5	1.6	0.15
Plasma insulin,				
d 51	2.33	2.34	0.076	0.99
d 113	6.65	5.98	0.076	0.54
d 187	5.06	4.16	0.076	0.41
d 261	9.62	6.16	0.076	< 0.01
Plasma IGF-I, ng/mL				
d 51	51.5	74.0	3.6	< 0.01
d 113	31.8	33.5	3.6	0.75
d 187	64.8	63.5	3.6	0.80
d 261	127.5	123.1	3.6	0.47

In the present experiment, heifers received corn-based supplement ad libitum for 50 d. Conversely, Scheffler et al. (2014) reported that steers fed a high-concentrate diet for 148 d after early weaning produced heavier carcasses with greater marbling scores compared to unsupplemented normal-weaned cohorts. Gasser et al. (2006) also observed that feeding a high-concentrate diet for 10 wk after early weaning hastened puberty attainment in beef heifers. In addition, heifers evaluated herein were still nursing, which likely alleviated concentrate intake during the imprinting period. Accordingly, Gasser et al. (2006) reported that earlyweaned heifer calves consumed approximately 2.5 to 3.0% of BW of a high-concentrate diet, whereas average concentrate intake herein was 0.83% of BW. Therefore, research is still warranted to determine if a longer period of imprinting to nursing beef heifers is required to further increase concentrate intake and effectively modulate fat accretion, metabolic responses, and reproductive development.

IMPLICATIONS

Providing a corn-based supplement ad libitum through a creep-feeder for 50 d to nursing heifers, as a method to stimulate the metabolic imprinting process (Lucas, 1991; Du



Figure 1. Puberty attainment of beef heifers receiving a combased supplement ad libitum through a creep-feeder for 50 d while nursing their damns (**MI**; n = 15), or cohorts that did not receive the supplement (**CON**; n = 15).

et al., 2010), did not hasten growth, fat accretion, or puberty attainment. Hence, additional research is still required, including different supplementation lengths, to further evaluate the impact of metabolic imprinting on growth and reproductive development replacement beef heifers.

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Effect of high sulfate water on behavior, performance, and volatile fatty acid production in lambs

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ABSTRACT: Sulfur-induced polioenchephalomacia (sPEM) is characterized by necrosis of the gray matter of the brain and is attributed to high dietary S and (or) S-compounds. We hypothesized that high sulfate (SO_4) water would impact behavior, performance, blood mineral metabolites, and volatile fatty acid (VFA) production in growing lambs. Growing lambs (n = 43; female = 22, male = 21; initial BW = 48.76 ± 16.44 kg) of Hampshire and Hampshire-cross breed types were randomly allotted to individual pens and acclimated to a forage-based pelleted diet. All lambs were provided drinking water with $3,043.89 \pm 746.11 \text{ mg SO}/L$ for a 28 d trial period. Feed and water disappearance were Lambs were also monitored daily for recorded daily. behavioral signs of S toxicity, including alertness and fecal consistency (as a measure of health), and were ranked using a daily ethogram including both behavior and water and feed intake data. The four highest ranking lambs were considered tolerant to high S, and the four lowest ranking lambs intolerant to high S. Plasma and rumen samples were obtained on d 0, 7, and 28, along with BW measures. Plasma samples were analyzed for blood mineral metabolites of Mo, Mg, Cu, and Fe. Performance measures included ADG, residual feed intake (RFI), and F:G. Rumen samples from the tolerant and intolerant lambs from each sampling day were purified and analyzed using a gas chromatograph for VFA concentrations. Results indicated that high SO₄ water affected (P < 0.001) ADG and F:G, as well as Zn concentration on d 28 (P = 0.04). Molar percentage of propionate was affected (P = 0.04) by the sampling day * tolerance class interaction; isobutyrate and valerate were affected (P < 0.01) by tolerance class; and isovalerate was affected (P = 0.01) by sampling day. This study suggests that administration of high SO, drinking water affects VFA concentrations in the rumen of sheep, potentially influencing animal health and performance.

Keywords: sheep, sulfur, volatile fatty acids

INTRODUCTION

As the most important nutrient, water is involved directly or indirectly in almost every physiologic process that is essential to livestock's well-being. 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). Ruminants are susceptible to health problems when high S content in water sources is encountered. The production of H₂S has been established as the mechanism of S toxicity, initiated by SO₄-reducing bacteria which typically use lactate as a carbon source and SO₄. Generation of acetate and S²⁻ from this process can diffuse into the blood stream across the rumen wall (Campbell and Postgate, 1965); additionally, H₂S gas can be eructated and re-inhaled by the animal (Gould et al., 2002). Tolerance to high S is variable depending on diet, supplementation, water, and environmental conditions (Raisbeck et al., 2007). Loneragan et al. (2001) reported that steers provided water with lower SO₄ levels (125 mg SO₄/L) gained BW faster and more efficiently than contemporaries administered high-S (500, 1000, or 2000 mg SO₄/L) water. Also, water sources with moderate to high levels of S combined with higher S feeds can exacerbate the condition, as this combination can cause animals to easily exceed safe dietary S requirements.

In addition to H_2S production, issues with high S can arise due to complexes formed between blood metabolites (Suttle, 1991). In particular, S, Mo and Cu form insoluble complexes and can prevent the uptake of Zn and Mo (Raisbeck et al., 2007). Also, Fe blood concentrations are indicative of the ability for Fe to bind to S, altering an animal's S status.

Finally, there is some evidence that changes in VFA production in the rumen may be associated with high dietary S. Beef cattle studies have demonstrated lower propionate and butyrate concentrations are associated with feeding of high S distillers grains (Saturi et al., 2013). Alternatively, Kung et al. (2000) and Smith et al. (2009) reported that high dietary S did not impact VFA concentrations in cattle. In sheep, Hegarty et al. (1994) reported an increase in total VFA concentration associated with a high S diet.

We hypothesized that high S water $(3,000 \text{ mg/L SO}_4)$ would negatively influence animal behavior and performance, while altering blood mineral metabolites and ruminal VFA production.

MATERIALS AND METHODS

Animal Care. All procedures were approved by the University of Wyoming Animal Care and Use Committee. The lamb trial occurred July 27^{th} to August 23^{rd} , 2013, at the University of Wyoming's Laramie Research Extension Center. Hampshire and Hampshire-cross growing lambs (n = 43; average initial BW 48.76 ± 16.44 kg) were randomly

allotted to individual pens in a confinement facility for a 28 d feed and water intake trial. Lambs were acclimated to the pelleted forage diet for 23 d prior to the start of the trial to allow for diet adjustment, and were also given a two week period to adjust to 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 once daily, *ad libitum*, and orts were weighed back each morning. On d 0, 7, and 28, 2-d BW were collected and averaged.

Water Sulfate. Sodium sulfate was mixed daily with water from the research facility (SO₄ = 77 mg/L) to create the desired level of 3,000 mg SO₄/L. The water mixture was tested daily during the first two weeks of SO₄ administration at the Wyoming Department of Agriculture Analytical Services (Laramie), and then every other day for the remainder of the study; actual levels were 3043.89 ± 746.11 mg SO₄/L. This level of SO₄ was chosen as previous experiments demonstrated that a level of 2,500 mg SO₄/L did not elicit significant health and performance changes in lambs (Jons, 2001).

Performance Measures. Average daily gain was determined by using initial and final 2-d average BW. The F:G ratio was estimated from average daily feed intake and ADG. Residual feed intake (RFI) was determined as the difference between actual feed intake and expected intake. Expected intake was calculated by regressing ADG and metabolic mid-weight (mid-weight^{0.75}) on actual feed intake (Cammack et al., 2005).

Animal Selection. Following the SO_4 treatment, "tolerant" and "intolerant" lambs were identified using a 2-tier selection process. Firstly, an ethogram (Table 1) was used to rate daily behavior during the 28 d trial. These daily behavior scores were averaged for each lamb, and lambs were ranked accordingly. Secondly, lambs on each tail of the behavior score distribution were considered for selection as tolerant and intolerant, respectively, based on their sex (and on ADG if the average behavior scores were similar), ultimately resulting in four tolerant lambs (two male; two female) and four intolerant lambs (two male; two female).

Plasma Mineral Metabolites. Blood samples were collected on d 0, 7, and 28 via the jugular vein for plasma mineral metabolite analysis. Minerals analyzed included Zn, Mb, Mg, Cu, and Fe. Day 0 served as the control for each animal. For mineral analysis, trichloroacetic acid (TCA) was used to precipitate the albumin and hemoglobin within the samples. Four standards and quality control (QC) tubes, one per every ten samples, were prepared using a Trace Element stock standard. One mL of each sample was pipetted into properly labeled tubes, and 3 mL of protein precipitating reagent was added to each sample. Both QC and standard tubes were then capped and vortexed for 5 min. All tubes were centrifuged for 10 min at 1,500 k x g; 1 mL of supernatant from each sample, QC, and standard was extracted and 3 mL of reagent water was added. Tubes were loaded onto the Autosampler (Agilent, Santa Clara, CA) and analyzed using the Agilent 7700 Inductively Coupled Plasma Mass Spectrometer (Agilent, Santa Clara, CA). The Agilent software program was used to monitor the output curves for quality and linearity.

Volatile Fatty Acids. Rumen samples were collected on d 0, 7, and 28 from all lambs. Samples from all collection days from the selected lambs, resulting in 24 samples, were strained and centrifuged to separate the fluid 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 was transferred to a vial for the gas chromatography. The supernatant in these vials were analyzed using the 6980n Network GC system (Agilent, Santa Clara, CA) for VFA concentrations.

Statistical Analysis. The GLM procedure of SAS (SAS Inst., Cary, NC) was used to determine the effect of water (and hence SO_4) intake on feed intake, ADG, F:G, and RFI using all animals in the trial. The CORR procedure was used to determine relationships between plasma mineral concentrations and water intake. Finally, the GLM procedure was used to determine effects of tolerance classification (tolerant or intolerant), sampling day (d 0, 7, or 28) and their interaction on VFA molar percentages. Post hoc analyses comparing treatment means were conducted with the LSMEANS procedure of SAS using a Tukey's adjustment and assuming an alpha of 0.05.

RESULTS AND DISCUSSION

No lambs demonstrated outward signs specific to sPEM. As anticipated, behavior, as assessed on a 1-5 scale (Table 1), was affected (P = 0.001) by average and total water intake (Table 2). Daily water intake and daily feed intake were not different ($P \ge 0.13$) in tolerant versus intolerant lambs. Both ADG and F:G were affected (P < 0.001) by average and total water intake. There were no differences in ADG (P = 0.64) between tolerance classes; however, F:G tended (P = 0.06) to be greater in tolerant lambs. Residual feed intake was affected (P = 0.04) by total water intake but not average daily water intake; additionally, tolerant lambs had greater (P = 0.01) RFI values than intolerant lambs, indicating, along with F:G, a greater efficiency associated with lower tolerance to high S.

Plasma Mo, Cu, Mg, Fe concentrations were not different $(P \ge 0.14)$ between neither tolerance classes nor sampling days. However, d 28 Zn concentrations were affected (P = 0.04) by water intake.

Propionate was affected (P < 0.04) by the interaction of sampling day and tolerance classification, with tolerant lambs having greater propionate molar percentage than intolerant lambs on d 0 only (Table 3). Sarturi et al. (2013) reported that steers on a high S diet (1.16% S DM basis) had lower propionate concentrations compared to steers on a low-S diet (0.82% S DM basis). Molar percentages of isobutyrate and valerate were greater (P < 0.01) in intolerant lambs than tolerant lambs (Table 4). Isovalerate was affected (P < 0.01) by sampling date, with d 7 molar percentage the greatest, d 28 molar percentage the least, and d 0 intermediate (Table 4). Uwituze et al. (2011) reported that steers on a high-S diet (0.65% S DM basis) had greater (P = 0.01) 3-methyl isovalerate concentrations than steers on a low-S diet (0.42% S DM basis).
Table 1. Ethogram of behavior for lambs.

Value	Behavior	Feed Intake ¹	Water Intake ¹
5	Normal – Skittish, alert	Normal	Normal
4	Slightly depressed – not as alert, still skittish	Normal	Normal
3	Slightly depressed – droopy ears, less reactive, poor overall appearance	Slightly decreased	Normal
2	Depressed – droopy ears, possible diarrhea, less responsive to stimuli, increased respiration	Decreased	Decreased
1	Sick – droopy ears, possible diarrhea, unresponsive to environment, little to no movement	Decreased to minimal	Decreased to minimal

¹Feed and water intake measured daily.

Table 2. Least squares mean behavior scores and performance trait measures for lambs administered drinking water with approximately 3,000 mg SO₄/ml.

Trait	Tolerant ¹	Intolerant ²	SEM
Behavior (scale 1-5)	4.88ª	4.51 ^b	0.300
Daily Water Intake (L/d)	6.79	6.29	2.569
Daily Feed Intake (kg/d)	2.47	2.24	0.626
RFI (kg/d)	0.11ª	-0.20 ^b	0.113
ADG (kg/d)	0.25	0.24	0.166
F:G (kg/kg)	1.31 ^y	1.25 ^x	0.029

¹Tolerant: Animals who scored an average of \geq 4.5 on the behavior scale over 28 d.

²Intolerant: Animals who scored an average of < 4.5 on the behavior scale over 28 d.

^{a,b}Least squares means within a row with different superscripts differ (P < 0.05)

^{x, y}Least squares means within a row with different superscripts tend to differ $(P \le 0.10)$

¹Feed and water intake measured daily.

Table 3. Interaction of tolerance classification \times sampling day least squares mean volatile fatty acid (VFA) molar percentages for lambs administered 3,000 mg SO₄/L water.

		Toler	ant ¹		Intolerant ²				P-value
VFA	d 0	d 7	d 28	SEM	d 0	d 7	d 28	SEM	Day x Tolerance
Acetate	62.36	62.26	62.55	1.010	61.85	60.92	63.31	1.010	0.59
Proprionate	21.93 ^{ac}	19.12°	19.39°	0.799	18.15 ^{bc}	19.55°	19.01°	0.799	0.04
Isobutyrate	0.75	0.84	0.63	0.106	1.09	1.09	0.80	0.106	0.72
Butyrate	12.85	15.66	15.54	1.208	16.66	16.12	15.00	1.208	0.20
Isovalerate	0.64	0.73	0.50	0.123	1.00	1.09	0.73	0.123	0.85
Valerate	1.47	1.40	1.40	0.093	1.26	1.23	1.15	0.093	0.90

¹Tolerant: Animals who scored an average of \geq 4.5 on the behavior scale over 28 d.

²Intolerant: Animals who scored an average of < 4.5 on the behavior scale over 28 d.

^{a,b}Least squares means within a row with different superscripts differ (P < 0.05).

Table 4. Tolerance classification and sampling day main effect least squares mean volatile fatty acid (VFA) molar percentages for lambs administered approximately $3,000 \text{ mg SO}_4/\text{L}$ water.

		Tolerance Class			Day		P-valu	<i>P</i> -values	
VFA	Tolerant ¹	Intolerant ²	SEM	0	7	28	SEM	Tolerance	Day
Acetate	62.39	62.02	0.583	62.10	61.59	62.93	0.714	0.66	0.43
Proprionate	20.15	18.90	0.461	20.04	19.33	19.20	0.565	0.07	0.54
Isobutyrate	0.74 ^b	0.99^{a}	0.061	0.92	0.96	0.71	0.075	0.01	0.07
Butyrate	14.68	15.93	0.697	14.75	15.89	15.27	0.854	0.22	0.65
Isovalerate	0.62	0.94	0.071	0.82 ^b	0.91ª	0.62°	0.087	0.08	0.01
Valerate	1.21 ^b	1.42ª	0.054	1.36	1.31	1.27	0.066	0.01	0.63

¹Tolerant: Animals who scored an average of \geq 4.5 on the behavior scale over 28 d.

²Intolerant: Animals who scored an average of < 4.5 on the behavior scale over 28 d.

^{a,b}Least squares means within a row with different superscripts differ (P < 0.05).

CONCLUSIONS

Results from this study indicate that drinking water containing approximately 3,000 mg SO₄/ml does not elicit signs specific to sPEM, but does affect growing lamb performance. Lambs identified as being tolerant demonstrated greater ADG and a greater F:G ratio than intolerant lambs. Sarturi et al. (2013) reported that steers fed a diet of 40% inclusion of 1.16% S, had a 15.6% decrease in ADG compared to steers on a diet of 40% inclusion of 0.82% S. The results from our study are in contrast with previous research in which the G:F ratio in feedlot steers decreased as water SO₄ levels increased (P < 0.01) (Loneragan et al., 2001). The relatively short duration of the intake trial in this study may not have been sufficient to elicit changes in ADG or feed intake observed in longer-term study, and may contribute to the conflicting result in feed efficiency.

In the rumen, Mo, Cu, and S can form thiomolybdate. Certain thiomolybdates have been detected in plasma, indicating that they can be absorbed across the rumen wall and could be possibly be indicative of Mo, Cu, and (or) S concentrations within the rumen (Spears, 2003). Moreover, S has been shown to reduce the bioavailability of Cu. One study suggested that Fe levels can substantially reduce a ruminant's Cu status (Spears, 2003), possibly being further indicators of S status in a ruminant. Results from this study showed no differences in Fe and Cu concentrations between tolerant and intolerant lambs on any of the sampling days. Sulfur has the ability to inhibit Zn uptake, which is further magnified when animals are on a high fiber diet (Raisbeck et al., 2007). Perhaps, Zn concentrations could be indicative of S levels.

Analysis of VFAs indicated some changes with sampling day, tolerance class, and their interaction. The literature for data associating changes in VFA production with high dietary S is limited. However, *in vitro* studies have demonstrated that DM S concentrations of 0.2, 0.4, 0.8% did not impact VFA (acetate, proprionate, isobutyrate, butyrate, isovalerate, and

valerate) molar percentages in cannulated Jersey steers (Smith et al., 2009). Kung et al. (2000) reported similar results, as 0.29 and 1.09% S on a DM basis did not alter the same VFA molar proportions in cattle. However, Zinn et al. (1997) reported that when S concentrations increased from 0.15 to 0.25% DM, molar percentage of acetate decreased but propionate percentage increased in feedlot cattle. These results taken together warrant further research into the potential of ruminal VFA production as an indicator of high dietary S.

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Effects of dietary urea concentration and zilpaterol hydrochloride on performance and carcass characteristics of finishing steers¹

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ABSTRACT: Cattle receiving zilpaterol hydrochloride (ZH) may recycle less N and require a greater supply of RDP. This study evaluated effects of ZH on performance and carcass characteristics of steers fed diets with increasing dietary RDP concentrations supplied as urea. Steers (n = 430; BW = 423 ± 4.5 kg) were sorted into 3 blocks according to BW and assigned to 1 of 6 treatments (6 pens per treatment) in a randomized complete block design. Treatments were a 2×3 factorial arrangement of either no ZH or ZH (75 mg ZH per steer daily) supplemented to finishing diets containing 0, 0.5, or 1.0% urea of dietary DM. Pen weights were recorded before treatment initiation; urea was fed for 27 d, and ZH treatments were fed for 24 d with a 3-d withdrawal period. Pen weights were recorded before transporting steers to a commercial abattoir. Continuous response variables were analyzed using linear mixed models and categorical data were analyzed using generalized linear mixed models. No ZH × dietary urea interactions ($P \ge 0.11$) occurred for all performance and carcass response variables. Increasing dietary urea linearly decreased (P = 0.01) ADG and DMI. A tendency for a linear decrease (P = 0.10) in HCW, and a tendency for a quadratic decrease (P = 0.07) in marbling score were observed as urea increased in the diet. Feeding ZH for the last 27 d (included a 3-d withdrawal period) of the finishing period increased (P < 0.01) ADG, decreased (P = 0.01) DMI, and increased (P < 0.01) 0.01) G:F compared with no ZH. In addition, ZH increased HCW (P < 0.01), dressing percentage (P < 0.01), LM area (P < 0.01), and yield grade (P = 0.02). Results indicate that cattle supplemented with ZH do not require additional RDP in the diet, and that performance and carcass characteristics were negatively affected when urea was increased in the diet.

Key words: degradable intake protein, steer, urea, zilpaterol hydrochloride

INTRODUCTION

Zilpaterol hydrochloride (**ZH**; Zilmax, Merck Animal Health, Summit, NJ) is a commercially available betaadrenergic agonist (β AA) that increases feedlot cattle performance. Previous research (Avendaño-Reyes et al., 2006; Vasconcelos et al., 2008) demonstrated that feeding ZH for the last 20 to 40 d of finishing increased ADG, HCW, dressing percentage, and LM area, but decreased 12th rib fat depth and marbling score. These changes in carcass characteristics typically result in increased red meat yield, with minimal changes on consumer acceptability despite increased Warner-Bratzler shear force (Hilton et al., 2009).

Greater animal performance associated with use of repartitioning agents, such as ZH, may alter livestock nutrient requirements (Reeds and Mersmann, 1991). Brake et al. (2011) demonstrated that urea-N recycled to the gut (as a percentage of total N intake) tended to be lower when ZH was included in corn-based diets fed to steers. This suggests that ZH may partition N into muscle protein synthesis, thus decreasing urea recycling. According to Titgemeyer et al. (2012), greater dietary N may be necessary to maintain rumen function when protein deposition in the body is increased.

We hypothesized that a greater dietary supply of ruminally available N would improve performance of feedlot cattle fed ZH. The objective of this study was to evaluate effects of ZH on performance and carcass characteristics of crossbred steers consuming diets with different concentrations of RDP supplied as urea.

MATERIALS AND METHODS

Receiving Cattle Management

All procedures were approved by the Institutional Animal Care and Use Committee at New Mexico State University. Twenty wk before the experiment, crossbred calves (n = 450) were shipped to the Clayton Livestock Research Center. Calves were weighed (228 ± 0.84 kg BW), vaccinated against viral and clostridial organisms (Vista 5 and Calvary 9, Merck Animal Health), treated for external parasites (Safe-Guard, Merck Animal Health), and given a growth implant (Revalor-IS, Merck Animal Health). Steers were then used for a 56-d immunology study (Graves et al., 2013). Metaphylaxis included tulathromycin (Draxxin, Zoetis Animal Health, Madison, NJ) or tildipirosin (Zuprevo, Merck Animal Health). Upon completion of the immunology

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study, steers were fed an 85% concentrate diet with monensin and tylosin (Elanco Animal Health, Indianapolis, IN) until approximately 155 d on feed.

Experimental Design and Dietary Treatments

At approximately 155 d on feed, 430 steers were weighed individually $(423 \pm 4.5 \text{ kg BW}; \text{ not fasting})$ in a hydraulic squeeze chute (Silencer, Moly Mfg. Inc., Lorraine, KS), reimplanted (Revalor-IS, Merck Animal Health), and sorted into 3 blocks according to BW. The average BW at time of sorting was 390 ± 1.1 , 424 ± 0.7 , and 454 ± 1.1 kg for blocks 1, 2, and 3, respectively. Within each block, steers were ranked according to BW and assigned to pens so that the average BW and SE were similar among pens of each block. There were 36 soil-surfaced pens (12×35 m, with 11 m bunk line) in 3 blocks with 12 pens per block and 11 to 12 steers per pen. Before the experimental period was initiated, all pens of cattle were fed a standard feedlot finishing diet with 0.5% urea (Table 1); ZH was not supplemented at this time.

The experiment was a randomized complete block design. Within each block, pens were randomly assigned to 6 dietary treatments in a 2×3 factorial arrangement. Treatments were either no ZH or ZH supplemented to finishing diets containing 0, 0.5, or 1.0% urea (Table 1). Dietary treatments were initiated after pens of cattle were weighed, 27 d before the scheduled harvest date. Treatments were fed for 24 d (due to beef processing plant availability) followed by a 3-d period for withdrawal from ZH. Cattle were fed twice daily, and ZH treatments were top-dressed directly onto the finishing diet. Before top-dressing, the appropriate amount of ZH for a pen of cattle was mixed with 200 g of wet corn gluten feed (Sweet Bran, Cargill Inc., Minneapolis, MN) in order to supply 75 mg ZH per steer daily. A rake was used in an attempt to distribute the wet corn gluten feed and ZH evenly with the finishing diet. The diet for pens of cattle receiving treatments with no ZH was top-dressed with 200 g of wet corn gluten feed only.

Feeding Management and Collections

Feed bunks were visually evaluated twice daily to determine the amount of feed to offer each pen of steers. Bunk management was designed to allow for little to no feed accumulation each morning. Feed was mixed in an overhead mixer (Butler Oswalt, Garden City, KS) and delivered to pens by a 6-bin feed truck with individual dispensing augers. Diet samples were collected weekly and analyzed for DM (100°C for 24 h in a forced-air oven) and other nutrients (Servi-Tech Labs, Amarillo, TX). Feed refusals were collected as needed and analyzed for DM to calculate daily DMI.

On the day of harvest, steers were moved from assigned pens to a platform scale for the recording of final BW. Steers were transported approximately 227 km to a commercial abattoir (Tyson Fresh Meats, Amarillo, TX). Steers were humanely harvested, and HCW and liver scores were recorded. Liver scores and measurements for carcass characteristics were collected by personnel from the Beef Carcass Research Center (West Texas A&M University, Canyon, TX). Carcasses were chilled for approximately 24 h and individual carcass measurements included marbling

Table 1. Ingredient and nutrient concentrations of dietary treatments fed to finishing steers

		Treatments	1
	0%	0.5%	1.0%
Item	Urea	Urea	Urea
Ingredient, % of DM			
Corn grain, flaked	60.99	61.01	60.95
Wet corn gluten feed ²	18.66	18.67	18.65
Corn stover	8.12	8.12	8.11
DDGS ³	9.35	9.82	8.91
Urea	-	0.49	0.98
Limestone	2.05	2.05	2.05
Beefmax 510 ⁴	0.062	0.050	0.062
Salt	0.25	0.25	0.25
Vitamin supplement5	0.002	0.002	0.002
Medicated supplement6	0.021	0.021	0.021
Nutrient Analysis ⁷			
CP, % DM	13.7	14.4	15.8
RDP ⁸ , % DM	7.3	8.4	9.7
RUP ⁸ , % of DM	6.3	6.0	6.1
Ca, % DM	0.81	0.73	0.76
P, % DM	0.46	0.42	0.47
NE ⁹ , Mcal/kg DM	2.12	2.21	2.21
NE ⁹ , Mcal/kg DM	1.50	1.52	1.51

¹Treatments were a 2 × 3 factorial arrangement of 2 levels of zilpaterol hydrochloride and 3 levels of RDP supplied as urea. ²Sweet Bran (Cargill Inc., Minneapolis, MN).

³DDGS = corn dried distiller's grains with solubles.

4Contained 1.8% Cu, 9.0% Zn, and 360 ppm Se (Cargill Inc.).

Supplied 770 IU vitamin A, 154 IU vitamin D, 5 IU vitamin E per kg of DM.

Supplied 34 mg monensin and 9 mg tylosin per kg of DM. (Elanco Animal Health, Indianapolis, IN)

⁷Based on proximate analysis (Servi-Tech Labs, Amarillo, TX).

⁸Calculated based on tabular values (NRC, 1996).

Calculated by Servi-Tech Labs.

score, USDA quality grade, 12th rib fat depth, LM area, KPH, and calculated yield grade. Dressing percentage was calculated by dividing the average HCW of the steers in the pen by the final BW of the pen.

Statistical Analysis

Performance and carcass data with continuous variables were analyzed using linear mixed models (SAS Inst. Inc., Cary, NC). Categorical data, such as liver scores and quality grades, were analyzed using generalized linear mixed models. The model included urea, ZH, and the urea \times ZH interaction, and block was random. Contrasts were used to test linear and quadratic responses of increasing urea concentrations. Initial and final BW were adjusted with a 4% shrink. Treatment differences were considered significant when $P \leq 0.05$.

RESULTS

Interaction of ZH and Dietary Urea

No interactions ($P \ge 0.24$) between ZH and dietary urea were observed for all performance response variables (Table 2). Similarly, no ZH \times urea interactions ($P \ge 0.11$) between were observed for carcass characteristics.

			Treatme	ents ¹					P-va	lue ²	
		No ZH			ΗZ			Urea	Urea		Urea
-	0%Urea	0.5%Urea	1.0%Urea	0%Urea	0.5%Urea	1.0%Urea	SEM	Lin.	Quad.	ΗZ	нz ×
Pens ³	9	9	9	9	9	9					
Performance											
Initial BW, kg ⁴	544	539	546	544	545	535	18.6	0.53	0.93	0.71	0.31
Final BW, kg	569	563	568	574	574	559	18.6	0.21	0.83	0.63	0.24
ADG, kg/d	0.92	16.0	0.83	1.11	1.09	0.90	0.06	0.01	0.22	<0.01	0.43
DMI, kg/d	8.93	8.87	8.39	8.45	8.44	7.61	0.39	0.01	0.15	0.01	0.75
G:F	0.10	0.10	0.10	0.13	0.13	0.12	0.01	0.11	0.68	<0.01	0.71
Carcass Characteristics											
HCW, kg	369	365	363	381	382	378	13.2	0.10	0.72	<0.01	0.71
Dressing %	64.8	64.8	63.8	66.4	66.5	67.7	0.60	0.82	0.95	<0.01	0.11
Marbling score	42.1	45.1	44.2	44.0	45.3	43.3	1.78	0.53	0.07	0.66	0.44
12 th rib fat depth, cm	1.15	1.34	1.25	1.27	1.23	1.22	0.11	0.73	0.30	0.98	0.20
LM area, cm ²	89.7	88.0	87.6	95.7	94.2	95.4	5.44	0.32	0.35	<0.01	0.71
KPH, %	1.86	1.81	1.82	1.82	1.87	1.77	0.03	0.16	0.47	0.76	0.19
Yield grade	2.64	2.86	2.78	2.56	2.61	2.49	0.17	0.75	0.18	0.02	0.52
Choice or better, %	56.0	66.0	58.0	63.4	59.2	57.2	9.07	0.72	0.45	0.98	0.50
Liver abscesses, %	20.4	17.3	13.0	10.4	9.92	19.6	5.41	0.77	0.57	0.28	0.14
Treatments (2 × 3 factorial	arrangement) we	re 2 levels of ZH	(no ZH vs. ZH to st	upply 75 mg zilpa	aterol hydrochlor	ide per steer dail	y) and 3 col	ncentrations	s of urea (0,	0.5, or 1.0%	6 urea) in
finishing diets (Table 1). Tr	eatments were fe	d for 24 d follow	ed by a 3-d withdra	wal period.							
$2 \Pi_{\text{rank}} \Pi_{\text{rank}} = D_{\text{rank}} \log 6 \omega $ the	linear affant of u	- I have Outed =	D value for the an	identia affiant af u	where $T \Pi = D_{-} value$	a far the main of	Wast of 7H.	I I voor 7 U	= D value i	for the inter-	antion of

Table 2. Effects of zilpaterol hydrochloride (ZH) and urea concentrations in finishing diets on performance and carcass characteristics of crossbred steers

²Urea Lin. = *P*-value for the linear effect of urea; Urea Quad. = *P*-value for the quadratic effect of urea; ZH = P-value for the main effect of ZH; Urea × ZH = P-value for the interaction of urea and ZH. ³Pens contained 11 to 12 steers. ⁴Initial BW = BW at the initiation of treatments (27 d before harvest).

Effects of Dietary Urea

Initial and final BW were not different ($P \ge 0.21$) among steers fed diets with 0, 0.5, or 1.0% urea (Table 2). Increasing urea in the diet linearly decreased both ADG (P = 0.01) and DMI (P = 0.01), but did not affect G:F ($P \ge 0.11$). Hot carcass weights tended to decrease linearly (P = 0.10) with increasing urea concentrations in the diet. Dressing percentage, 12th rib fat depth, LM area, KPH, yield grade, percentage of carcasses grading Choice or better, and incidence of liver abscesses were not affected ($P \ge 0.16$) by increasing concentrations of urea in the diet. A tendency for a quadratic decrease (P =0.07) in marbling score was observed as the concentration of urea increased in the diet.

Effects of ZH Supplementation

Initial and final BW were not different ($P \ge 0.63$) for steers fed ZH compared with no ZH (Table 2). Cattle fed diets with ZH had greater (P < 0.01) ADG, lower (P = 0.01) DMI, and greater (P < 0.01) G:F. Steers receiving diets containing ZH exhibited greater HCW (P < 0.01), dressing percentage (P < 0.01), LM area (P < 0.01), and yield grade (P = 0.02) compared with steers receiving diets with no ZH. Feeding ZH in the diet did not affect ($P \ge 0.28$) marbling score, 12th rib fat depth, KPH, percentage of carcasses grading Choice or better, or incidence of liver abscesses.

DISCUSSION

Interaction of ZH and Dietary Urea

We hypothesized that a greater dietary supply of ruminally available N would improve performance of feedlot cattle receiving ZH. This hypothesis was based on evidence that greater protein accretion in response to ZH may reduce urea recycling to the rumen as a result of decreased AA catabolism by the liver (Brake et al., 2011). In this study, finishing diets contained 0, 0.5, and 1.0% urea (DM basis) to supply an estimated 7.3, 8.4, and 9.7% RDP (DM basis), respectively (Table 1). According to Cooper et al. (2002), 8.3% RDP is considered adequate in typical feedlot diets with no BAA. Therefore, it was assumed that diets containing 0.5% urea supplied adequate rumen available N, whereas rumen available N was potentially deficient in diets containing 0% urea, and in excess in diets containing 1.0% urea. Lack of interaction between ZH and dietary urea for performance and carcass traits suggests that feeding ZH did not require a greater dietary supply of ruminally available N. Despite evidence that ZH could reduce urea recycling (Brake et al., 2011), alterations in N metabolism were not enough to increase RDP requirements above 8.4%.

Effects of Dietary Urea

Increasing urea in the diet decreased animal performance regardless of ZH application. The decrease in ADG is likely due to lower dietary energy intake as a result of decreases in DMI with increasing dietary urea. The greatest numerical decreases for both DMI and ADG occurred when urea was increased from 0.5% to 1.0% of the diet. The decrease in DMI

in response to increasing dietary urea is in contrast to Shain et al. (1998) and Gleghorn et al. (2004), but consistent with Milton et al. (1997). In these studies, dietary urea remained constant throughout finishing. Furthermore, diets with the greatest urea concentrations had dietary CP (and perhaps RDP) concentrations similar or lower than the intermediate diet (0.5% urea) in this study. In the current study, cattle were fed a finishing diet containing 0.5% urea (and no ZH) until 27 d before harvest, at which time the diet was replaced with a treatment diet containing either lower (0%), similar (0.5%), or greater (1.0%) concentrations of urea (Table 1). It is possible that an abrupt change from the diet containing 0.5% urea to the diet containing 1.0% urea negatively affected DMI. Therefore, decreases in both DMI and ADG when urea was increased from 0.5% to 1.0% of the diet in this study are perhaps due to an excess supply of ammonia.

A tendency for HCW to decrease with increasing dietary urea is likely associated with decreases in performance as described above. These results contrast Milton et al. (1997), Shain et al. (1998), and Gleghorn et al. (2004), who observed either no difference or increased HCW with increasing dietary urea. However, in the aforementioned studies, the feeding protocol remained uniform throughout, perhaps resulting in more favorable performance and thus improved HCW. In this study, other carcass measurements were not affected by dietary urea, and are in agreement with the results of Shain et al. (1998) and Gleghorn et al. (2004).

Effects of ZH Supplementation

Final BW were not different, but steers fed ZH for 24 d followed by a 3-d withdrawal period had 16% greater ADG, 6% lower DMI, and 30% greater G:F compared with steers receiving no ZH. These results agree with previous studies (Vasconcelos et al., 2008; Holland et al., 2010), although Scramlin et al. (2010) reported greater final BW for cattle fed ZH. In a review, Mersmann (1998) explains that synthetic β AA may traverse the blood-brain barrier and influence central nervous system-mediated responses such as satiety and hunger signals, which may explain decreases in DMI of cattle fed ZH.

Feeding ZH increased HCW by 15 kg, which is similar to results of Montgomery et al. (2009) and Scramlin et al. (2010). Also, a 3.7% greater dressing percentage for cattle fed ZH agrees with results of Vasconcelos et al. (2008). In this study, no differences in marbling score, 12th rib fat depth, or KPH suggest that ZH did not affect carcass fat distribution. Results observed for 12th rib fat depth and KPH are consistent with the findings of Montgomery et al. (2009) and Holland et al. (2010). Vasconcelos et al. (2008) indicated that decreases in marbling are observed when fat deposition is decreased as a result of ZH. However, this study reported greater ADG than the present study, which may have affected body composition. Larger LM area in response to feeding ZH is indicative of increased protein deposition, and consistent with the findings of Avendaño-Reyes et al. (2006). Additionally, ZH improved yield grade which agrees with results reported by Holland et al. (2010). This suggests that ZH more directly affected protein deposition, as carcass fat composition was not altered by inclusion of ZH. Because body fat composition was similar between ZH treatments, there was no difference in quality grade. In contrast, Montgomery et al. (2009) and Vasconcelos et al. (2008) observed an increase in select carcasses, which can be explained by decreased marbling score when ZH was used, a response not reported in the present study. Zilpaterol hydrochloride did not increase condemned livers, which agrees with Holland et al. (2010), but may have been a result of including tylosin in the diet.

IMPLICATIONS

Results imply that cattle supplemented with zilpaterol hydrochloride do not require additional ruminally degradable protein in diets to maximize performance. Providing excess ruminally available nitrogen decreased performance of feedlot cattle during the last 27 days of the finishing period, regardless of zilpaterol hydrochloride. This contrasts with our hypothesis that a greater dietary supply of ruminally available nitrogen would improve performance of feedlot cattle fed zilpaterol hydrochloride. Regardless of dietary urea concentration, feeding zilpaterol hydrochloride improved performance, hot carcass weight, dressing percentage, and longissimus muscle area, and did not decrease carcass fat deposition to the same extent reported previously.

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Effects of juniper species and stage of maturity on nutritional, digestive, and plant secondary compound characteristics

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ABSTACT: Rising feed costs and reoccurring feed shortages necessitate the investigation of alternative feeds. Nutritional characteristics of Juniperus species are either unknown or limited to leaves and ground material from small stems. Thus, the objective of this study was to quantify nutritional characteristics, 48-h true IVDMD (tIVDMD), microbial gas production, and secondary compound characteristics of entire woody plant material of four Juniperus species: J. pinchotii (J. pin); J. monosperma (J. mon); J. virginiana (J. vir) at immature and mature stages of growth. Immature plants had greater CP concentrations (P < 0.04) vs. mature plants regardless of species. Immature plants had less (P < 0.04) NDF and ADF concentrations vs. mature plants, except for J. vir. Immature J. mon, J. pin, and J. ash had the least (P <0.001) amount of NDF and ADF vs. immature J. vir. In general immature J. mon and J. pin plants had greater (P < 0.02) tIVDMD, total 48-h and asymptotic gas production vs. mature contemporaries. Immature J. mon and J. pin plants had greater (P < 0.001) tIVDMD vs. immature J. vir and J. ash plants. In general total 48-h and asymptotic gas production was greatest (P < 0.001) for immature J. mon, J. pin, and J. ash vs. immature J. vir. Condensed tannins (CT) were greatest (P < 0.02) in immature plants of J. pin, J. mon, and J. ash vs. their mature contemporaries, differences in CT concentrations of immature species were also detected (P < 0.04). Volatile oil yields were unaffected by maturity and species with the exception of mature J. vir having greater (P < 0.02) volatile oil than other mature species. Labdane acids were negligible in J. pin, J. ash, and J. vir, whereas J. mon had significantly greater (P < 0.001) total labdane acids. Concentrations of labdane acids in J. mon may indicate abortifacient risk in pregnant cattle. In relation to research where J. pin was successfully fed, nutritional similarities of J. ash, J. vir, J. mon to J. pin, clarify their potential as effective roughage ingredient alternatives.

Key words: juniper, nutritional quality, secondary compounds

INTRODUCTION

Drought-induced feed shortages and the subsequent rise in feed costs justifies investigation into alternative feed stuffs in an effort to provide cost effective alternatives to livestock producers. Woody plants from the genus *Juniperus* infest millions of acres in the southern plains and southwestern U.S., adversely affecting rangeland health and productivity. However, this increasingly abundant plant biomass is now being utilized as a roughage ingredient in mixed diets for sheep; using either leaves (Whitney and Muir, 2010) or leaves and small stems (Whitney et al., 2014).

Utilization of woody-biomass as a feed resource is not entirely novel nor is it limited to certain geographic regions; however, research with these non-traditional feeds receives greatest consideration during times of feed shortages and high feed costs, but diminishes when feed related inputs and feed availability stabilizes (NRC, 1983).

Secondary compounds present in *Juniperus* species such as volatile oil and condensed tannins (CT) can either positively or negatively affect the animal and rumen function. Preliminary research regarding the feeding value of ground juniper plants is promising, but information is limited to ground *J. pinchotii* and leaves and small stems (< 3.6 cm). Widespread utilization of multiple juniper species (beyond *J. pinchotii*) requires baseline information regarding their nutritional, *in vitro* digestive, and plant secondary characteristics, in addition to whether the entire plant biomass can be utilized. Therefore, the objective of this study was to compare nutritional characteristics of *J. pinchotii, J. monosperma, J. ashei* and *J. virginiana*) at mature and immature growth stages.

MATERIALS AND METHODS

Study Design and Harvesting Protocol

Juniper was collected over a 4 week period in early spring. Juniperus species were harvested at 4 separate geographic locations. Juniperus pinchotii was collected in Tom Green County, Juniperus ashei was collected in Edward County, TX Juniperus virginiana was collected in Bastrop County, TX and Juniperus monosperma was collected in Torrance County, NM. At each of the four sites, 4 plots, separated by a minimum of 180 m were designated as harvest sites. All plots for each species were maintained separate throughout the entirety of the trial, as plot was the experimental unit. One mature male and one mature female tree (height: > 3 m), and two male and two female immature plants (height: 1 to 1.8 m) from each plot were mechanically harvested and transported to a central location and processed within 72 hr. Material was then chipped and fine-ground through a hammermill to pass a 4.76-mm sieve and dried at 55°C in a forced-air oven for 48 h, and finally ground through a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 2-mm screen, and stored at -20°C.

Laboratory Analysis

Dry matter of ground juniper subsamples was calculated by drying the 4.76-mm hammermilled material at 105°C in a forced-air oven for 24 h. Nitrogen was analyzed by a standard method (AOAC, 2006) and CP calculated as 6.25 \times N. The NDF and ADF were analyzed sequentially according to Van Soest et al. (1991), modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, NY), correcting for residual ash, and using α -amylase, and Na sulfite. Lignin and ash were analyzed by a standard methods (AOAC, 2006). The protocol for collecting rumen fluid was approved by the Texas A&M University Institutional Animal Care and Use Committee. Rumen fluid from sheep (n = 4) fed a lowquality basal hay diet and 125 g of a 12% CP supplement, was collected via oral lavage into pre-warmed thermoses, purged with CO₂, filtered through 2 layers of cheese-cloth, combined, and continuously purged with CO₂ until added to pre-warmed gas production modules. For each jar, 56 mL McDougal's buffer solution (1.0 g of urea L^{-1}) and 14 mL of RF was added to 0.7 g of juniper material. Jars were flushed with CO₂, Ankom[™] gas production modules secured, and then incubated for 48 h at 39°C. All species and maturities within plot were analyzed in duplicate; thus, 4 separate gas runs were evaluated. In addition, 2 blanks were used in each run and treated similarly to other jars but did not contain a feed substrate. After 48 h, undigested feed material was rinsed out of each jar and analyzed by NDF procedures according to Van Soest et al. (1991) for true IVDMD (tIVDMD). In addition, undigested material was subsequently analyzed for N to determine NDIN. The (CT) in each juniper species and maturity classification were assayed for soluble, proteinbound, and fiber-bound fractions by methods described by Terrill et al. (1992). Oven dried samples were then freezedried and standards prepared for each Juniperus species. Air-dried samples were steamed distilled to yield values for total volatile oil content. Isocupressic (ICA), agathic (AGA), imbricatoloic (IMB), and dihydraogathic (DHAA) acids were analyzed at the USDA-ARS Poisonous Plants Research Center, Logan UT according to methods described in Gardner and James (1999).

Statistical Methods

Data were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Dry matter, ash, NDF, ADF, lignin, CP, tIVDMD, NDIN, volatile oil, condensed tannins, isocupressic acid, agathic acid, imbricatoloic acid, and dihydraogathic acids were analyzed using a model that included species × maturity. Experimental unit was plot and data are reported as least squares means with greatest pooled SEM.

Kinetic analysis of cumulative gas production was evaluated using several nonlinear functions (Schofield and Pell, 1995) and nonlinear fitting performed using GasFit (http://nutritionmodels.tamu.edu/gasfit.html) as previously described (Tedeschi et al., 2009). Results indicated the Gompertz 2-pool nonlinear function had the lowest sum of square of errors for most of the variables and therefore was chosen:

$$Y = a \times exp \left(-exp \left(1 + b \times (c - t)\right)\right) + d \times \left(-exp \left(1 + e \times (c - t)\right)\right)$$

where Y is gas produced, mL; a is the asymptote, mL; b is the fractional degradation rate h⁻¹; t is time after starting incubation, h; c is lag time, h; d is the asymptote of the second pool (assumed to be fiber), mL; and e represents fractional degradation rate of the second pool, h⁻¹. Parameters calculated from GASFIT were analyzed using PROC MIXED with a model that included species × maturity. Run was the random variable and each plot was evaluated on separate days; thus, *in vitro* run = plot. Correlations among nutritional characteristics and gas production characteristics were evaluated by species and maturity using Pearson correlation.

RESULTS AND DISCUSSION

Crude Protein and Neutral Detergent Insoluble Nitrogen

Crude protein and NDIN is summarized in Table 1. An effect (P < 0.04) of maturity on CP was observed in all species. The CP values in the current study are less than those observed by Whitney et al. (2014) and likely a result of greater woody biomass, especially in the mature juniper plants. In the current study, CP concentrations of both mature and immature plants are comparable to other low-quality roughage sources i.e., CSH (5% CP; NRC 2007), oat straw (4% CP; NRC, 2007), wheat straw (3% CP; NRC, 2007), and pine bark (1% CP; Min et al., 2012). No differences (P =0.23) in NDIN were observed between species or maturities.

NDF, ADF and ADL

Immature plants had significantly less (P < 0.01) NDF, ADF, ADL concentrations than mature plants, with the exception of J. virginiana and J. ashei (Table 1). In general J. pinchotii and J. monosperma, possessed the least NDF, ADF, and ADL fiber concentrations followed by J. ashei and J. virginiana. Differences in fiber components between plant species and maturity found in the current trial may be the result of greater leaf to stem ratio and the horizontal (shrub-like) vegetative growth structure of J. monosperma, J. pinchotii, and J. ashei compared to J. virginiana. Average fiber components of mature juniper species in the current study (NDF = 66%, ADF = 55%), although greater than immature juniper species, are comparable to or less than the other low-quality roughage ingredients, i.e., CSH (NDF= 87%, ADF= 68%; NRC, 2007), wheat straw (NDF= 81%, ADF= 58%; NRC, 2007), poplar bark (NDF = 63%, ADF= 52%; Gharib et al., 1975), and pine bark (NDF = 78%, ADF = 72%; Min et al., 2012).

Gas Production and 48-h true IVDMD

Gas production and 48-h tIVDMD data is summarized in Table 2. *In vitro* 48-h gas production data can provide valuable information in regards to forage digestibility (Schofield and Pell, 1995). Total gas production correlated with tIVDMD across all species (0.95, P = 0.001) and negatively correlated with NDF (r = -0.97, P = 0.01), and ADF (r = -0.97, P =0.001), yet these correlations were most pronounced amongst immature *Juniperus* species. Immature *J. pichotii*, *J. pinchotii*, and *J. ashei* produced greater total gas than mature plants of the same species (P < 0.02). Asymptotic gas production of immature *J. pinchotii and J. ashei* was also greater than mature plants of the same species (P < 0.07). A greater proportion of cell wall components and greater lignin concentrations would be indicated by the decreased asymptotic gas production in mature plant species (Blummel et al., 1997). Comparing immature species, asymptotic and total gas production was lowest in J. virginiana (P < 0.03; Table 2). The most accurate correlative time point value for in vivo digestibility of low-quality feed ingredients (e.g., straw) with the gas production technique has been measured between 45 to 52 h of in vitro fermentation (Prasad et al., 1994). Consistent with current findings from Cornou et al. (2013), the most repeatable and reproducible measures using ANKOM gas production modules were asymptotic and 48-h gas production. Although VFA production was not measured in the current study, Doane et al. (1997) observed a strong positive relationship between total gas production and VFA production and that both of these were linearly related to NDF digestion. Utilization of the entire plant biomass in the current study resulted in an expected decrease in tIVDMD compared to J. pinchotii leaves (67%, Whitney and Muir 2010) and leaves and small stems (55%, Whitney et al., 2014). Notwithstanding a decrease in expected tIVDMD associated with using the entire plant bio-mass, digestibility

Table 1. Comparison of plant species and stage of maturity's effect nutritional characteristics (DM basis) of *J. pinchotii*, *J. monosperma*, *J. ashei*, and *J. virginiana*¹

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	J. pin	chotii	J. monos	sperma	J. (ashei	J. virg	giniana	Pooled
Item, % ²	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	SEM
DM	68.1 ^{ab}	69.1 ^a	66.7 ^{a,bc}	65.8 ^{bc}	64.9°	65.4 ^{bc}	61.5 ^d	62.2 ^d	0.70
Ash	5.7 ^{ab}	4.3 ^{cd}	5.4 ^b	4.4 ^{cd}	5.9 ^a	4.7°	4.1 ^d	3.6°	0.30
NDF	50.1°	66.9 ^{ab}	50.0°	64.6 ^{bc}	54.4 ^d	67.4 ^{ab}	62.6 ^c	68.5 ^a	1.32
ADF	40.7 ^{cd}	56.2 ^{ab}	40.0 ^d	54.0 ^b	44.2°	55.5 ^{ab}	52.9 ^b	58.0 ^a	1.46
ADL	21.1°	25.0 ^{cd}	23.1 ^d	26.3 ^{bc}	29.3 ^a	29.8 ^a	27.9 ^{ab}	29.1ª	0.75
CP	4.7 ^a	3.6 ^{cd}	4.6 ^{ab}	3.6 ^{cd}	4.1 ^{bc}	3.4 ^d	4.6 ^a	3.7 ^{cd}	0.18
NDIN	1.54 ^{ab}	1.44 ^b	1.80 ^a	1.67 ^{ab}	1.60 ^{ab}	1.35 ^b	1.56 ^{ab}	1.64 ^{ab}	0.23
Oil	0.60^{ab}	0.46 ^{bcd}	0.50 ^{abc}	0.43 ^{bcd}	0.30 ^d	0.40 ^{cd}	0.47 ^{bcd}	0.66 ^a	0.05
ECT	5.5 ^a	3.1 ^b	4.1 ^a	2.5 ^b	3.8 ^a	2.7 ^b	2.8 ^{bc}	2.7°	0.29
FCT	1.2 ^{ab}	0.59 ^b	0.71 ^b	0.59 ^b	$1.8^{\rm a}$	0.82 ^{ab}	1.1 ^{ab}	1.3 ^{ab}	0.39
PCT	1.6 ^{bc}	1.1°	1.4 ^{bc}	1.1°	3.5 ^a	2.2 ^b	1.7 ^{bc}	1.5 ^{bc}	0.37
CT	8.4 ^a	4.7 ^{bc}	6.3 ^b	4.2°	9.0 ^a	5.7 ^{bc}	5.6 ^{bc}	5.5 ^{bc}	0.72

¹Items within row with a different superscript differ, P < 0.05 and data are least squares means. Juniper species × stage of maturity. Imm = Immature growth stage (1 to 1.8 m); Mat = mature growth stage (> 3 m).

²Oil = total volatile oil; ECT, PCT, FCT, CT = extractable, protein-bound, fiber-bound, and total condensed tannins respectively.

Table 2. Effects of plant stage of maturity on *in vitro* fermentation dynamics of *J. pinchotii*, *J. monosperma*, *J. ashei*, and *J. virginiana*¹

	J. pin	nchotii	J. monos	sperma	J. as	shei	J. virgi	niana	Pooled
Item, % ²	Imm	Mat	Imm	Mat	Imm	Mat	Imm	Mat	SEM
tIVDMD%	49.8 ^a	29.7 ^c	49.4 ^a	33.0 ^e	43.6 ^b	30.0 ^c	33.6°	29.1°	1.67
Total, mL	44.5 ^a	25.7°	45.2 ^a	25.7°	43.2 ^a	31.0 ^{bc}	32.3 ^b	24.9 ^c	2.16
a, mL	26.8 ^a	10.6 ^c	23.7 ^{ab}	17.7 ^{bc}	27.6 ^a	17.1 ^{bc}	15.5 ^{bc}	16.9 ^{bc}	2.82
b, h ⁻¹	0.17 ^b	0.46^{a}	0.31 ^{ab}	0.16 ^b	0.14 ^b	0.15 ^b	0.17 ^b	0.15 ^b	0.07
c, h	0.08^{ab}	0.10^{ab}	0.10 ^{ab}	0.23 ^{ab}	0.19 ^{ab}	0.30 ^a	0.09 ^{ab}	0.02^{b}	0.08
d, mL	20.7 ^a	21.6 ^a	21.9 ^a	10.2 ^b	19.9 ^a	18.8 ^a	18.8 ^a	14.9 ^{ab}	2.41
e, h ⁻¹	0.30 ^b	0.18 ^b	0.32 ^b	1.5 ^a	0.12 ^b	0.89 ^{ab}	0.21 ^b	0.15 ^b	0.38

¹Items within row with a different superscript differ, P < 0.05 and data are least squares means. Juniper species × stage of maturity. Imm = Immature growth stage (1 to 1.8 m); Mat = mature growth stage (> 3 m).

¹tIVDMD = 48-h true IVDMD; Total = 48-h cumulative gas production (mL/g substrate DM); a = asymptote (mL/g substrate DM); b = fractional degradation rate; c = lag time; d = asymptote of second pool (mL/g substrate DM); e = fractional degradation rate of second pool.

values appear comparable to digestibility of other lowquality feed ingredients, e.g., CSH (21 to 32%; Torrent et al., 1994; Whitney and Muir, 2010) and wheat straw (27 to 41%; Haddad et al., 1994). Extrapolating fiber and *in vitro* digestibility characteristics from feeding trials conducted with *J. pinchotii* (Whitney et al., 2014) suggest that both mature and immature *J. monosperma* and *J. ashei* could also be utilized as an effective roughage ingredient alternatives.

Secondary Compounds

Condensed tannin concentrations are summarized in Table 1. Witihin J. pinchotii, J. monosperma, and J. ashei species, CT was less (P < 0.02) in mature vs. immature plants). Differences in total CT amongst immature species was observed (P = 0.04) with immature J. pinchotii and J. ashei having the greatest CT concentrations, whereas no differences (P = 0.26) were observed for mature plants. Total CT for immature J. pinchotii was 8.4%, which is greater than the 6% CT found in ground leaves and stems (< 3.6 cm) reported by Whitney et al. (2014). These CT concentrations are slightly less than pine bark (10%; Min et al., 2012) and similar to serice alespedeza (Solaiman et al., 2010). Condensed tannin intake has been shown to enhance growth performance in goats consuming sericea lespedeza (6.4% CT; Solaiman et al., 2010) and improve rumen fermentation in goats consuming a mixed diet containing pine bark (10% CT; Min et al., 2012), suggesting that optimal percentage of dietary CT intake may fall within the range of 2 to 4% (Solaiman et al., 2010; Min et al., 2012), however, this may vary based on CT contained in the respective plant species. Results from Whitney et al. (2014) also suggest an optimal CT intake level may exist, due to greater growth performance of lambs consuming 11 to 19 g CT/kg of DM in a mixed diet. Total CT concentrations from the 4 Juniperus species in the current study suggest that both immature (5.6 to 8.4% CT) and mature (4.2 to 5.7% CT) plants, when used in a mixed diet containing 30% ground juniper material, would provide approximately 12 to 25 g/kg of CT from ground juniper plants. When comparing mature species J. virginiana had the greatest (P < 0.02) volatile oil vs. the other mature species, whereas only a tendency (P = 0.06) was observed amongst immature species. Volatile oil concentrations in dried ground juniper material in the current study measured significantly less than fresh J. ashei leaf material (0.5% vs. 2.5% oil; Adams et al., 2013), J. pinchotii leaf material (0.5% vs. 1.0%; Adams et al., 2010), and J. virginiana (0.7% vs. 2.3%; Animut et al., 2004). The reduction in volatile oil yields is noteworthy when discussing the merits of Juniperus as feed ingredient as the volatile oil from processed plant material is much less than fresh material. Thus, direct comparisons with previous studies involving animal consumption of fresh Juniperus foliage vs. processed (ground and dried) Juniperus material is of limited value.

Labdane Acids (ICA, AGA, IMB, DHAA)

The labdane resin acid isocupressic acid (ICA) and similar related labdane acids including agathic acid (AGA),

imbricatoloic acid (IMB), and dihydroagathic acid (DHAA), were analyzed in the four Juniperus species, and are discussed as a percentage of DM. Concentrations of ICA in ponderosa pine and its related metabolite AGA, which is found in J. osteosperma and J. occidentalis, may cause late-term abortions in cattle (Gardner et al., 2010; Welch et al., 2011). Labdane acid concentrations over 0.5% DM are potentially abortifacient, with greatest risk at > 1.0% DM (Gardner et al., 1994; Welch et al., 2013). Similarly IMB and DHAA are suspected to be biologically active abortifacient compounds (Welch et al., 2013). Juniperus ashei, J. virginiana, and J. pinchotii contained negligible amounts of ICA, IMB, and DHAA ($\leq 0.01\%$ DM). Similarly, minor concentrations of AGA were measured in J. ashei (0.02% DM), virginiana (0.02% DM), and pinchotii (0.05% DM) with no differences (P < 0.18) between immature and mature growth stages. Thus, results suggest J. ashei, J. virginiana, and J. pinchotii pose little abortifacient risk. In contrast, J. monosperma had greater (P < 0.05) concentrations of ICA, AGA, IMB, and DHAA compared to J. ashei, virginiana, and pinchotii. Furthermore, immature J. monosperma plants vs. mature plants had greater (P < 0.05) concentrations of ICA (0.27 vs. 0.16%); IMB (0.19 vs. 0.10%), DHAA (1.4 vs. 0.71%), and AGA (0.20 vs. 0.16%). Although ICA and AGA concentrations individually fall below the conservative 0.5% threshold, total combined labdane acid concentrations in J. *monosperma* may pose a risk for late term abortions in cattle, and needs to be further evaluated (Welch, 2013).

IMPLICATIONS

Findings indicate that immature and mature J. pinchotii, J. monosperma, and J. ashei possess nutritional characteristics that when extrapolated to sheep feeding trials, indicate suitability as a roughage ingredient. With the exception of J. virginiana, similarities in nutritional characteristics across mature juniper species indicate that although significantly lower in quality than immature Juniperus plants, are comparable to many currently approved roughage ingredients. Additional research feeding each respective mature species in mixed diets is warranted to determine specific feeding applications; i.e., ruminant species, stage of production and dietary inclusion rate. Plant secondary compound characteristics (CT, volatile oil yield, and labdane acids) provide valuable baseline information that helps extrapolate results to feeding trials that have used J. pinchotii. However, bioactivity of CT and volatile oil in each species deserves further investigation. Combined levels of ICA, AA, IMB, and DHAA in J. monosperma warrant additional research into its suitability as a feed ingredient for pregnant cattle.

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Evaluation of titanium dioxide as a digestibility marker for horses

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ABSTRACT: Our hypothesis was that titanium dioxide (TiO₂) could be used as an external marker to predict fecal DM output and DM digestibility in horses. The objective of our study was to compare values of fecal output obtained by total fecal collection compared to fecal DM output predicted by TiO₂ in two age groups of horses fed an alfalfa-based diet. To test our hypothesis, twelve stock-type horses were used comprising two age groups: mature (average age 10.3 +4.1 y; n = 6) and senior (average age 21.5 + 1.9 y; n = 6). Horses were housed individually in 3.7 m \times 3.7 m stalls and allowed ad libitum access to water and mineral block. Basal diet consisted of alfalfa hay (22.8% CP and 32.7% NDF; DM basis) and was fed at 1.9% of BW as fed basis. The experiment was conducted in 2 20-d periods, d 1 to 10 allowed for adaptation to basal diet and corn-based pelleted supplement. On d 1 to 9, the pelleted supplement contained no TiO₂. Baseline values of Ti in horse feces were assessed using a sample collected from each horse on d 10 prior to feeding the pelleted supplement with TiO₂. Beginning on d 10, 5 g of TiO₂ was dosed via the pelleted supplement (0.7)kg twice daily; as fed basis). Horses were fitted with a fecal collection bag during the final 5 d of each period. For each day of fecal collection, feces from each horse were collected every 6 h for 24 h. Additionally, rectal grab samples were collected on d 16 to 20 at 12 h intervals with collection times advancing by 2 h each day. Comparisons were made using a completely randomized block design and the GLM procedure of SAS. Horse was experimental unit and model included fecal output estimation method for baseline corrected and uncorrected samples. Correction for baseline resulted in no difference between fecal DM output and DM digestibility estimations methods (P=0.17). However, when samples were uncorrected for baseline fecal DM output was underestimated by 45% compared to actual (P < 0.001) resulting in an overestimation of DM digestibility by 59% (P < 0.01). These data confirm our hypothesis that TiO₂ can potentially be used as a digestibility marker in horses. However, the elevated levels of Ti in the fecal samples without TiO₂ is concerning and warrants further investigation.

Key words: Horses, Digestibility Markers, Titanium Dioxide

INTRODUCTION

Forage intake and diet digestibility in many species can be difficult to measure directly because direct measurements involve housing animals in confinement to collect fecal output and limiting diet selection options. Additionally, total fecal collections in horses are time-consuming, expensive, and can only utilize geldings which severely limits the ability of research to impart meaningful results for the entire industry. The use of inert markers added to diets allow for the prediction of diet digestibility without the need to quantitatively estimate feed intake and fecal output. Chromic oxide (Cr_2O_3) has been used extensively as an external marker in digestion trials with ruminants. However, Cr_2O_3 is problematic due to concerns over potential carcinogenic properties and health hazards associate with inhalation (Titgemeyer et al., 2001).

Titanium dioxide (TiO₂) has been used as an alternative digestibility marker to Cr₂O₂ in cattle and sheep (Titgemeyer et al., 2001; Myers et al., 2006; Glindemann et al., 2009), pigs (Jagger et al., 1992) and chickens (Short et al., 1996). Although TiO₂ has not been validated in horses it is currently being utilized (Winsco et al., 2013). In dairy cows, Hafez et al. (1988) concluded TiO, has a 99% fecal recovery rate and Titgemeyer et al. (2001) found that fecal recovery averaged 93% when fed with forage and grain to beef steers. Little data has been published to validate the use of TiO₂ as an external digestibility marker in horses. We hypothesized that TiO₂ could be used as an external marker to predict fecal DM output and DM digestibility in horses. The objective of our study was to compare values of fecal output obtained by total fecal collection compared to fecal output predicted by TiO₂ in two age groups of horses fed an alfalfa-based diet.

MATERIAL AND METHODS

All procedures for the following experiment were approved by the New Mexico State University Institutional Animal Care and Use Committee.

Experimental design and treatments

Twelve stock-type horses were used in a completely random design. Prior to the beginning of the study, horses were dewormed and dentation was checked and any abnormalities found were corrected. Fecal DM output was determined by total fecal collection with fecal bag or TiO₂ level in a composite sample made from rectal grab samples collected every 3 h for 24 h. There were two age groups: mature (average age 10.3 + 4.1 y; n = 5) and senior (average age 21.5 + 1.9 y; n = 6). Horses were fed (1.9% of BW as fed basis) a basal diet consisting of alfalfa hay (22.8% CP and 32.7% NDF; DM basis) and allowed ad libitum access to a trace mineral block (American Stockman Big 6 Trace Mineral Salt Block, Tractor Supply, Las Cruces, NM). Horses were housed individually within 3.7 m \times 3.7 m stalls in a semi-enclosed barn. Stalls were equipped with automatic water fountains and bedded with wood shavings. Due to stall limitations, horses were randomly assigned to 1 of 2 groups and the experiment was conducted in 2 20-d periods. Days 1 to 9 allowed for adaptation to basal diet and pelleted supplement for delivery of TiO₂. Prior to TiO₂ dosing, the pelleted supplement contained 90% fine ground corn and 10% molasses (DM basis) and a portion of the fine ground corn was replaced with to TiO₂ make the TiO₂ pellet. Beginning on d 10, 5 g of TiO, was dosed twice daily via the pelleted supplement (0.7 kg twice daily; as fed basis). The pelleted supplement containing TiO, was offered each day prior to offering the basal diet. Consumption of the pelleted supplement was rapid with no refusals.

Horses were fitted with a fecal collection bag during the final 5 d of each period. For each day of fecal collection, feces from each horse were collected every 6 h for 24 h. Feces from each horse was weighed individually and after mixing by hand, a 10% subsample was preserved. Additionally, samples retrieved from the rectum were collected on d 15 to 20 at 12 h intervals with collection times advancing by 2 h each day. For example, samples were collected on d 15 at 0600 h and 1800 h, d 16 at 0800 h and 2000 h, d 17 at 1000 h and 2200 h, d 18 at 0000 h and 1200 h, d 19 at 0200 h and 1400 h, and d 20 at 0400 h and 1600 h to cover a 24 h period. All fecal samples were froze at -200 C immediately for later analysis. Fecal samples were weighed and dried at 500 C in a forced-air oven for 48 h, allowed to air-equilibrate and weighed again. Dried samples were ground through a 1 mm screen (Wiley Mill, Thomas Scientific, New York, NY) then composited into a representative 24 h sample based on total fecal collection or hour of sampling. Titanium from the feces was extracted according to the wet-ash method of Myers et al. (2004) and quantified using a microtiter plate reader (BioTek Instruments Inc., Winooski, VT) at a wavelength of 410 nm. Baseline values of Ti in horse feces were assessed using a sample collected from each horse on d 10 prior to feeding the pelleted supplement with TiO₂.

Calculations and statistical analysis

Comparisons were made using a completely randomized design and the GLM procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC). Horse was used as the experimental unit. Model included fecal output estimation method and age of horse for baseline corrected and uncorrected samples. Age of the horse was not significant (P = 0.57) so it was dropped from the model. Means were calculated using LSMEANS. Treatment effect was considered significant when the probability of a greater F was < 0.05. When F-tests were significant, mean separations were performed using PDIFF and standard error calculated using STDERR.

RESULTS AND DISCUSSION

To our knowledge use of TiO, as a marker of fecal DM output and DM digestibility has not been validated in horses. Data for fecal DM output and DM digestibility are shown in Table 1. Correction for baseline (Ti concentration in feces prior to dosing) resulted in no difference between fecal DM output and DM digestibility estimations methods (P = 0.17). However, when samples were uncorrected for baseline fecal DM output was underestimated by 45% compared to actual (P < 0.001) resulting in an overestimation of DM digestibility by 59% (P < 0.001).

Baseline fecal samples were analyzed to determine if there were substances present that could interfere with the detection of Ti. Baseline samples in our study averaged 0.85 + 0.11 g/kg DM Ti. Glindemann et al. (2009) reported that sheep fed a forage diet had a mean equivalent of 0.24 g/kg DM Ti in feces without the addition of TiO₂ using the method of Brandt and Allam (1987). The higher values found in our study compared to Glindemann et al. (2009) may be due to differences in animal (ovine vs. equine), diet (grass vs. legume) or differences in analytical method for Ti. We used the wet-ash method of Myers et al. (2004) which yielded higher recoveries of Ti compared to the dry-ash technique

	Treat	ment ¹		
	Measured Calculated		SEM	P -value
Uncorrected ²				
Fecal DM output, kg/d	2.40	1.52	0.10	< 0.001
DM digestibility, %	66.5	74.9	1.21	< 0.001
Corrected ³				
Fecal DM output, kg/d	2.40	2.78	0.20	0.17
DM digestibility, %	66.5	65.9	2.41	0.86

Table 1. Fecal outputs and DM digestibilities for horses consuming alfalfa hay at 1.9% of BW.

¹Measured = total fecal DM output and DM digestibility determined using total fecal collections; calculated = total fecal DM output and DM digestibility was calculated based on titanium dioxide output ²Values are not corrected for titanium dioxide in fecal samples collected prior to titanium dosing. ³Values are corrected for titanium levels in fecal samples collected prior to titanium dosing.

used by Glindemann et al. (2009). When the background Ti was subtracted, Ti recoveries were closer to 100% and estimation of fecal DM output and DM digestibility did not differ from total collection with fecal bags (P = 0.17). However, caution should be exercised and further research is needed to determine the source of the Ti in the feces without TiO₂ added. The baseline sample that was used for this experiment was collected once it is questionable that this concentration will remain stable over time. Furthermore, the high background Ti in the baseline fecal sample without TiO₂ could not be conclusively addressed to diet composition, soil contamination, or housing.

IMPLICATIONS

According to the results of our study digestibility estimates obtained with TiO_2 may be inflated due to background interference in horse feces. In our study DM digestibility was overestimated by 59% with TiO_2 when values were not corrected for background interference in the Ti assay. This could lead to inadequate diet formulation that could have consequences in horse production.

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Effects of controlled body weight gain on growth and reproductive performance of heifers grazing native rangeland during the pre-breeding period

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ABSTRACT: Forty-eight Angus \times heifers (initial BW = 230 \pm 1.26 kg), grazing native range (7.9% CP, 65.2% NDF, DM basis) were used in a completely randomized experiment and assigned to one of three treatments, 56 d prior to breeding. Treatments were: 1) Dried distillers grains plus solubles (DDGS), fed at 1.81 kg heifer-1 d-1 to provide 0.36 kg/d of gain for 28 days, then fed at 0.68 kg heifer-1 d-1 to provide an ADG of 0.18 kg/d for another 28 d (HL); 2) DDGS fed to accomplish the low rate of gain (0.18 kg/d) for the first 28 days, then the increased rate of gain (0.36 kg/d) for another 28 d (LH); 3) DDGS fed to provide an ADG of 0.27 kg/d throughout the feeding period, which equaled the average expected gain for the LH and HL treatments (MOD). Heifers were weighed every 14 d, at which time, blood samples were taken via coccygeal venipuncture and serum progesterone concentrations were evaluated. Heifers were synchronized at the end of the feeding period, using a CoSynch+CIDR (progesterone) protocol in conjunction with fixed-time AI. Heifers received an injection of GnRH (100 µg, i.m.) and a CIDR containing 1.38 g of progesterone was inserted at d-7 relative to timed AI. At d 0, the CIDR was removed and heifers subsequently received 25 mg of PGF2a. Heifers were observed for estrus and then bred 12 h after confirmation of estrus. Any heifer not bred by hour 54 after removal of CIDR was given GnRH (100 µg, i.m.) and AI. Heifers were placed with bulls for 60 d. There was no difference ($P \ge 0.13$) in BW and ADG across treatment. Likewise, percent pubertal prior to breeding and conception rate in heifers did not differ ($P \ge 0.66$) amongst weight gain groups. Therefore, based on these data the onset of puberty and subsequent reproduction status was not dependent on timing or rate at which these heifers acquire body weight, as long as growth is seen in the pre-breeding period.

Key words: Growth, Heifer, Reproduction

INTRODUCTION

One of the most challenging aspects of a beef cattle operation is heifer development. Heifer management programs should be developed with the goal of reaching a certain BW prior to breeding to ensure reproductive success (Patterson et al., 1992). This target breeding weight was thought to be 60-65%. However, (Funston and Deutscher, 2004; Roberts et al., 2009) have suggested that this number could be around 58% of mature BW, without impacting reproduction. Lesmeister et al. (1973) indicated that lifetime productivity of a heifer is improved if she is able to give birth early. Decreasing the age at which heifers become pubertal will allow for greater opportunity to cycle prior to breeding, granting them more opportunity to conceive earlier; this is substantiated by (Byerley et al., 1987; Hare and Bryant, 1985), who indicated that females bred on their pubertal estrus had lower pregnancy rates, when compared to those bred at subsequent estrus. Onset of puberty is associated with nutrition, as determined by Schillo et al. (1992), who demonstrated that plane of nutrition is inversely proportional to age at onset of puberty, indicating that nutrition plays a role in reproductive processes. This is expanded on by Freetly et al. (2001), who suggested that altering post-weaning gains could be used to optimize feed resources and that as long as heifers grow to meet minimum body weight prior to breeding, that growth following weaning may be altered, with no adverse effects in reproduction. We hypothesized that a higher plane of nutrition early in the prebreeding phase would yield higher percentages of heifers attaining puberty prior to breeding and increase pregnancy rates in a timed-artificial insemination program. The objectives of the study were to determine if modifying growth patterns during the development period would influence growth, onset of puberty, and conception rates in heifers grazing native rangelands in the arid southwest.

MATERIALS AND METHODS

Animals, Diets and Treatments

All experimental procedures were reviewed and approved by the New Mexico State University Institutional Animal Care and Use Committee. Forty-eight Anguscrossbred heifers (initial BW = 230 ± 1.26 kg) were used in a completely randomized experiment. Heifers were randomly assigned to one of three treatments designed to provide controlled ADG. Specifically, treatments were: 1) Dried distillers grains plus solubles (DDGS), fed at 1.81 kg heifer-1 d-1 to provide 0.36 kg/d of gain for 28 days, then fed at 0.68 kg heifer-1 d-1 to provide an ADG of 0.18 kg/d for another 28 d (HL); 2) DDGS fed to accomplish the low rate of gain (0.18 kg/d) for the first 28 days, then the increased rate of gain (0.36 kg/d) for another 28 d (LH); 3) DDGS fed to provide an ADG of 0.27 kg/d throughout the feeding period, which equaled the ADG for the LH and HL treatments (MOD). Treatments ceased upon initiation of the breeding season. Heifer's grazed native

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range pastures (7.9% CP and 65.2% NDF, DM basis). Pastures (two pastures per treatment) consisted of predominantly blue grama (Bouteloua gracilis) and wolftail (Lycurus phleoides) as described by Forbes and Allred (2001) and were supplemented in bunks, three times per week. Heifers were provided 14 d to adapt to DDGS prior to the initiation of the experiment. Heifers were weighed every 14 d until breeding and given free choice access to a loose trace mineral (Corona Range & Livestock Research Center mineral, Hi-Pro Feeds, Friona, TX; NaCl >38.7%, P >8%, Mg >2%, K >2%, Zn >2,000 ppm, Mn >2,500 ppm, Cu >2,000 ppm, Se >13 ppm, Vitamin A >54,420 units/kg). Blood Collection and Assay Blood samples were collected via coccygeal venipuncture every 14 d and analyzed for serum progesterone concentrations. Blood was collected in 7-mL sterile serum separator tubes (CORVAC, Tyco Healthcare Group LP, Mansfield, MA) and allowed to clot for 2 h until centrifugation at 1,500 x g for 30 min at 4°C. Serum was decanted and then stored at -20°C until assays could be conducted. Serum progesterone concentrations were quantified by RIA, in duplicate, using components of a solid phase kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA; Coat-A-Count) as reported by Schneider and Hallford (1996). Intra assay CV was 4.1%. Pubertal status at breeding was determined by two consecutive serum progesterone concentrations above 1 ng/mL (Byerley et al., 1987). Blood collection via coccygeal venipuncture occurred 30 d post conception.

Breeding

Heifers were synchronized at the end of the feeding period using the Co-Synch + CIDR (progesterone insert) protocol with fixed-time AI. Heifers received an injection of GnRH (100 µg, i.m.) and a CIDR containing 1.38 g of progesterone was inserted at d-7 relative to timed AI. At d 0, the CIDR was removed and heifers subsequently received 25 mg of $PGF_{2\alpha}$. Heifers were observed for estrus and bred 12 h after confirmation of estrus. Any heifer not bred by 54 h after removal of CIDR was given GnRH (100 µg, i.m.) and AI. Heifers were placed with bulls for 60 d. Pregnancy and first service conception rates were confirmed with calving dates. First service conception rates were calculated as the percentage of heifers diagnosed as pregnant 30 d after initiation of breeding season. This was confirmed by serum analysis using the BioPRYN testing service, which measures the presence of Pregnancy-Specific Protein B (Integrated Dairy Services, Inc, Clovis, NM).

Laboratory Analysis

Forage samples were collected during breeding, to assess the composition of the native pastures used for the experiment. Samples collected were dried for 72 h at 55°C in a forced-air oven (Blue M Electric Company, Blue Island, IL) and ground using a 2-mm screen (Wiley Model 4, Thomas Scientific, Swedesboro, NJ). Ground samples were then analyzed for DM and ash (AOAC, 1990), (LECO FP-528, Leco Corp., St. Joseph, MI), and NDF using an ANKOM 200 fiber analyzer (ANKOM Technology, Fairport, NY). Nutrient analysis of DDGS was determined by a commercial laboratory (SDK Laboratories, Hutchinson, KS).

Calculations and Statistical Analysis

All data were analyzed as a completely randomized design using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Pasture was the experimental unit and the model included effects of treatment. When a significant (P < 0.10) treatment effect was detected, LS means were separated using possible pairwise comparisons using the PDiff option of SAS. Reproductive traits were analyzed using the PROC FREQ procedure of SAS.

RESULTS AND DISCUSSION

Growth Performance

Forage quality was similar among all pastures. No difference (P = 0.99) in BW was observed after the first 28 d of the experiment (Table 1). There tended to be a difference for d 0-28 ADG (P = 0.11) with MOD being greater than HL or LH. Interestingly, ADG across all treatments was greater than our expected values in the first 28 d. It is believed that this could be attributed to compensatory gain, which may have resulted from moving these heifers from lower quality forage onto slightly higher quality native range pastures for the initiation of this study. On days 28-56 ADG did not differ (P = 0.60) and it was observed to decrease regardless of assigned treatment compared to the first 28 d. The LH treatment group had the lowest ADG, despite receiving the greatest amount of DDGS. We attribute this decrease in ADG, for the LH treatment, to low annual precipitation, resulting in a decline in forage production during this time (<100 kg of DM/kg in ach pasture by the end of the study). Potentially, this led to limited intake. No difference (P = 0.50) in overall ADG as observed from d 0-56. Overall dietary S (water, forage, and DDGS) was calculated to be 0.36%, which is below the maximum tolerable concentration associated with cattle, which is 0.4% (NRC, 2000) It is not fully understood why performance was not as expected between treatments despite the different amounts of diet being provided. Clanton et al. (1983) while performing a similar experiment indicated that heifers fed for maintenance gained more than predicted based on NRC calculations. Additionally, there is the potential that during the adaptation period at the start of the study caused a subclinical S toxicity within the rumen, causing similar performance amongst groups. However, there were no outward signs of S toxicity.

Puberty & First Service Conception Rates

Treatment had no effect (P = 0.66) on onset of puberty. In this experiment, 49% (n = 23) of the total number of heifers (n = 48) became cyclic prior to breeding. First service conception rates for HL, MOD and LH were 62.5%, 71.4% and 62.9% respectively. Despite the numerical difference amongst groups, treatments did not differ (P = 0.71). This is supported by Freetly et al (2001), who indicated that as long as heifers meet a minimum body weight prior to breeding, that growth patterns may be changed in the post-weaning period with no adverse impacts on reproduction. Our findings also align with Lynch et al. (1997), who demonstrated that heifers fed to achieve varying rates of gain during the development phase had similar overall pregnancy rate.

Item ¹	MOD	HL	LH	SE ²	P-value
BW, kg					
d 0	231	231	226	1.28	0.13
d 28	268	268	268	5.03	0.99
d 56	283	280	271	5.97	0.47
ADG, kg/d					
d 0-28	1.19	0.98	0.96	0.06	0.11
d 28-56	0.32	0.48	0.23	0.15	0.60
d 0-56	0.76	0.73	0.60	0.09	0.50
First service conception rate ³ , %	71.4	62.5	62.9	0.08	0.71

Table 1. Effect of programmed rate of body	weight gain of beef heifers	grazing native rangelands	and supplemented
dried distillers grains plus solubles			

¹Treatments: 1) Dried distillers grains plus solubles (**DDGS**), fed at 1.81 kg·heifer⁻¹·d⁻¹ to provide 0.36 kg/d of gain for 28 days, then fed at 0.68 kg·heifer⁻¹·d⁻¹ to provide an ADG of 0.18 kg/d for another 28 d (**HL**); 2) DDGS fed to accomplish the low rate of gain (0.18 kg/d) for the first 28 days, then the increased rate of gain (0.36 kg/d) for another 28 d (**LH**); 3) DDGS fed to provide an ADG of 0.27 kg/d throughout the feeding period, which equaled the ADG for the LH and HL treatments (**MOD**). Study lasted 56 d, on d 28, diets changed to achieve our secondary ADG goals for each treatment group.

 $^{2}n = 2.$

³First service conception rate: calculated as a percentage of all heifers confirmed pregnant 30 d after initiation of the breeding season.

IMPLICATION

Timing of gain and rate of gain did not affect subsequent production or reproduction. Due to the limited number of heifers involved in this experiment, more work is needed to evaluate the impact on rate and timing of gain with regards to heifer development, onset of puberty, pregnancy rates and subsequent calving.

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Chemokine ligand twelve (CXCL12) protein in ovine placenta increases during early gestation: role in maternal-fetal crosstalk?

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ABSTRACT: Chemokine ligand twelve (CXCL12) and its receptor, chemokine receptor four (CXCR4) may promote implantation and placentation during early gestation in sheep. Our laboratory demonstrated enhanced CXCL12 and CXCR4 mRNA and protein expression in maternal (caruncle) placenta and fetal membranes (FM) at specific days of early pregnancy in sheep. This chemokine signaling axis may therefore be an important regulator in fetal membrane tissue to promote fetal survival. However, to further elucidate functionality of the CXCL12/CXCR4 signaling axis, it is pertinent to localize CXCL12 in sheep uterine tissue. We hypothesized that CXCL12 will be localized in FM and maternal placenta and CXCL12 expression will increase at the time frame of fetal attachment. Therefore, our objectives were to localize CXCL12 using immunofluorescence and determine level of expression based on staining intensity evaluated using image analysis in ovine placental tissues during early gestation. Uteri with intact FM were collected from crossbred Western Range ewes (n = 3 per d) on d 18, 20, 22, 24, 26, and 28 after mating. A cross-section of uterine tissue was used for immunofluorescent detection of CXCL12 protein followed by image analysis to determine staining intensity. Chemokine ligand twelve was localized in the uterine luminal epithelium, intercaruncular glandular epithelium and FM on each day of gestation. Greater CXCL12 protein immunoreactivity (P <0.05) was present in FM tissue on d 22 compared to d 18, and 28 as well as on d 24 compared to d 18, 20, and 28 of gestation. The increase of CXCL12 expression in FM suggests that CXCL12 plays a role in communication at the fetal-maternal interface. Because CXCL12 promotes proper invasiveness in an autocrine manner and stimulates cell proliferation in human trophoblast cells, we suggest CXCL12/CXCR4 signaling is playing a role in maternal-fetal communication and possibly contributing to fetal attachment and subsequent placentation. Analyzing intensity of CXCL12 staining and localization in uterine and fetal tissue of ruminants during early gestation provides new insights into potential functions of CXCL12/CXCR4 signaling during attachment and placental development.

Key words: CXCL12, CXCR4, implantation, placentation

INTRODUCTION

Chemokine ligand twelve (CXCL12) and its receptor, chemokine receptor four (CXCR4) were originally found to

be vital for generation of B-cell lymphopoiesis (Nagasawa et al., 1996). The CXCL12/CXCR4 signaling axis may also promote attachment and invasion of fetal membrane tissues (FM) into the maternal endometrium and increase angiogenesis to stimulate formation of a functional placenta (Tachibana et al., 1998; Dominguez et al., 2003). We observed increased CXCL12 and CXCR4 mRNA and protein in ovine FM and uterine caruncle (CAR) tissue during the timeframe of fetal attachment and placental development (Quinn et al., 2014). Fetal growth relies on proper fetal attachment and placental development to regulate exchange of nutrients and respiratory gases (Faber and Thornburg, 1983). Intrauterine growth restriction (IUGR) is a major concern in the livestock industry, leading to impaired embryo growth and development (Reynolds et al., 2013). Because IUGR is directly related to poor utero-placental blood flow, it is important to determine the function of specific proteins that regulate angiogenesis of the placenta. Vascular endothelial growth factor (VEGF) is a key regulator of placental vascularization (Charnock-Jones et al., 2004; Borowicz et al., 2007). Our laboratory observed increased mRNA for VEGF, its two receptors (VEGFR) and CXCL12, and CXCR4 on d 25 and/or 30 of pregnancy compared to d 20 in ovine FM. Expression of VEGF was also greater (P < 0.05) in ovine trophectoderm (OTR) cells treated with 25 and 100 ng/mL of CXCL12 compared to control (Quinn et al., 2014). In endothelial cells, VEGF induces CXCR4 and CXCL12 production and CXCL12 in turn enhances VEGF expression, thus establishing a positivefeedback loop (Salvucci et al., 2002). To enhance our understanding of CXCL12 functions during early gestation, it is important to determine the localization of CXCL12 in sheep uterine tissues. We hypothesized that CXCL12 will be localized in FM and maternal placenta and CXCL12 expression will increase at the time frame of fetal attachment. Therefore, our objectives were to localize CXCL12 using immunofluorescence and determine level of expression based on staining intensity evaluated using image analysis in ovine placental tissues during early gestation.

MATERIALS AND METHODS

Animals and Tissue Collection

All procedures involving animals were approved by North Dakota State University Institutional Animal Care and Use Committee. Procedures were previously published by Grazul-Bilska et al. (2010, 2011). Briefly, uteri were obtained from crossbred Western Range (primarily Rambouillet, Targhee, and Columbia) ewes (n = 3 per d) on d 18, 20, 22, 24, 26, and 28 after mating (day of mating = d 0). At tissue collection, specimen pins were inserted completely through the uterus and FM at the level of the external intercornual bifurcation to maintain specimen morphology. Cross sections of the entire gravid uterus (~0.5 cm thick) were obtained using a Stadie–Riggs microtome knife followed by immersion in 10% neutral buffered formalin for 48 h and paraffin embedded according to standard histological procedures (Luna, 1968).

Immunohistochemistry

Paraffin-embedded tissues were sectioned at 5 µm, mounted onto glass slides, and de-paraffinized with a histologic clearing agent (Histoclear; National Diagnostics, Atlanta, GA, USA) and series of rehydration ethanol washes (100%, 95%, 70%, 50% ethanol, respectively). Antigen retrieval was performed in 10 mM sodium citrate buffer pH = 6 with 0.05% Tween 20 in a 2100 retriever (Electron Microscopy Sciences, Hatfield, PA, USA), and each slide was then rinsed twice in Tris-buffered saline with Triton X-100 (TBST; 0.05 M Tris, 0.15 M NaCl, 0.1% TritonX-100). To block nonspecific binding of antibodies, each slide was treated for 20 min with blocking buffer (10% normal goat serum). Tissue sections were incubated with specific primary antibody for CXCL12 (1:50 dilution in 1X TBS; monoclonal mouse, R&D Systems, Minneapolis, MN, USA) and incubated overnight with constant movement at 4° C. The slides were protected from light and incubated for 1 h at room temperature with a 1:200 dilution of Alexa647 labeled secondary antibody (goat anti-mouse, Invitrogen A21235, Grand Island, NY, USA). Each slide was mounted with Pro-Long Gold with 4,6-diamidino-2-phenylindole (DAPI; Life Technologies, Grand Island, NY, USA) to counterstain nuclei. Photomicrographs were taken at the same exposure time with Zeiss Imager.M2 epifluorescence microscope using 10x objective and a AxioCam HRm camera, as well as a Zeiss piezo automated stage controlled by MosaiX module of Zeiss AxioVision software (Carl Zeiss Microscopy, LLC; 1 Zeiss Dr., Thornwood, NY, 10594, USA). Using this method we obtained mosaic images of large tissue areas containing a whole cross-section of the uterus. Control sections were incubated with normal goat serum in place of CXCL12 primary antibodies.

Image Analysis and Statistical Analysis

The MosaiX images of the trophoblast cells in each sample (n = 3 ewes per d) were analyzed using the Fiji Is Just Image J software program (Schindelin et al., 2012). The background from each image was subtracted and ten separate areas of CXCL12 intensity in the trophoblast cells was measured for each ewe. The mean immunoflorescent signals, SD and SEM of the trophoblast cells for three ewes per d of gestation were calculated. Significant differences in CXCL12 trophoblast cell intensity were determined at P < 0.05 using

an unpaired two-tailed Student's t-test by Prism (version 5 from GraphPad Software, Inc.).

RESULTS AND DISCUSSION

Implantation in ruminants consists of three stages; 1) preattachment in which the conceptus elongates, 2) an apposition stage, and 3) an adhesion stage, when development of the placenta occurs (Igwebuike, 2009). These stages happen on d 15 to 25 in sheep and are critical to establishment of pregnancy (Bazer et al., 2012). In uterine tissue, CXCL12 was localized to the uterine luminal epithelium, intercaruncular glandular epithelium, and FM on each day of gestation (Fig. 1). Because of the specific localization that was observed, we suggest that CXCL12 may function in communication at the maternalfetal interface. Since CXCL12 promotes proper invasion of FM in an autocrine manner and suppresses apoptosis in human trophoblast cells, it likely has similar functions in sheep (Jaleel et al., 2004). Localization of CXCL12 in uterine luminal epithelium and FM could indicate that CXCL12 promotes fetal attachment and survival.

Uterine glands secrete proteins, such as growth factors into the uterine lumen essential for fetal development (Reynolds et al., 1998; Gray et al., 2001). The CXCL12/ CXCR4 signaling axis stimulates synthesis and secretion of angiogenic factors such as VEGF and FGF2 (Rosenkilde and Schwartz, 2004). The specific localization of CXCL12 in intercaruncular glandular epithelium suggests involvement of CXCL12 in the secretion of growth factors into the uterine lumen, thereby promoting placental development. Future co-localization studies of CXCL12 and VEGF could provide more insight into how these two factors contribute to vascularization of the placenta. Chemokine ligand twelve staining intensity in FM increased (P < 0.05) on d 22 compared to d 18, and 28 as well as on d 24 compared to d 18, 20, and 28 of gestation (Fig. 2). Similar CXCL12 protein (determined using Western immunoblot) increase was observed in FM on d 25 of gestation compared to d 20 (Quinn et al., 2014). The increase on d 22 and 24 suggests that CXCL12 signaling is supporting maternal-fetal communication and possibly contributing to fetal attachment and subsequent placentation.

Further, CXCL12 promotes recruitment of NK cells (CD16) into human decidual tissues and Treg cells positive for CXCR4 migrate into the uterus of pregnant mice in response to CXCL12 (Lin et al., 2009). Therefore, CXCL12 may also provide an optimal immunological environment at the maternal-fetal interface.

IMPLICATIONS

Methodology used in this study emphasized the importance of image analysis to study fetal-maternal interactions during early gestation using immunofluorescence, and provided improved methods for future studies by other animal scientists. Few studies have localized CXCL12 in sheep uterine tissue. We have demonstrated that CXCL12 is specifically localized to FM, and uterine luminal and glandular epithelium. Therefore, it may play a role in maternal-fetal



Figure 1. Representative image of chemokine ligand twelve (CXCL12) localization in sheep uterine tissue on d 22 of gestation. A cross section of uterine tissue with fetal membrane intact was imaged at the same exposure time using MosaiX imaging. Chemokine ligand 12 is localized in the uterine luminal epithelium (white bar), uterine glands (white arrow head) and trophoblast cells (white arrow) of pregnant sheep. Yellow fluorescence denotes CXCL12 localization and blue represents 4,6-diamidino-2-phenylindole (DAPI) counterstaining of nuclei.

communication, specifically during implantation and initiation of placental development. Determining the functionality of CXCL12 based on our localization studies is now underway. Deciphering the function of CXCL12 may lead to future therapeutic targets for early gestational complications. These therapeutics will be advantageous to the livestock industry by potentially decreasing embryo mortality and improving birth weights.

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Figure 2. Representative MosaiX images of chemokine ligand twelve (CXCL12) localization in sheep uterine tissue on d 18, 20, 22, 24, 26, and 28 of gestation. A cross section of uterine tissue with fetal membrane intact was imaged at the same exposure time using MosaiX imaging. The intensity of trophoblast cell (white arrows) staining was evaluated using Fiji Is Just Image J software and analyzed using Prism (version 5 from GraphPad Software, Inc.). On d 22, and 24, CXCL12 protein levels increased (P < 0.05) in trophoblast cells compared to d 18 and 28 and d 18, 20 and 28 respectively. Different letters above each bar denote a significant difference. Yellow fluorescence represents CXCL12 and blue signifies 4,6-diamidino-2-phenylindole (DAPI) counterstaining of nuclei.

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Days of Gestation

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Effects of ad libitum supplement containing increasing levels of microalgae, *Scenedesmus sp.*, on site and extent of digestion in beef heifers consuming a forage-based diet

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INTRODUCTION

ABSTRACT: 1 The use of feedstuffs containing a high level of ash, such as the microalgae, Scenedesmus sp., could serve as an intake limiter for cattle offered free choice supplements on rangelands. Four Angus heifers (avg BW 409 \pm 18kg) fitted with ruminal and duodenal cannulas were used in a 4 \times 4 Latin square. Heifers were provided ad libitum access to chopped (2.54 cm) Sorghum × Sudan hay (8.8% CP and 71% NDF, OM basis) and one of four pelleted dietary treatments: 1) ad libitum access to soybean meal based supplement (37.9% Soybean hulls, 53.0% Soybean meal, 9.1% molasses; CON); 2) ad libitum access to a supplement formulated to contain Scenedesmus sp. (microalgae) at 17% of DM (33.3% soybean hulls, 17% microalgae, 37.7% Soybean meal, 8.7% molasses, DM basis; 17%); 3) ad libitum access to a supplement formulated to contain 42% microalgae (30.3% soybean hulls, 42% Microalgae., 18.9% Soybean meal, 9.1% molasses, DM basis; 42%); 4) ad libitum access to a supplement formulated to contain 66% microalgae (24% soybean hulls, 66% microalgae, 9% molasses, DM basis; 66%). Supplements were formulated to be isonitrogenous. The trial consisted of four 21 d periods with 17 d for diet adaptation and 4 d of intensive sampling. Increasing levels of microalgae had no effect on forage OM intake (P = 0.13) however a cubic effect (P = 0.01)was observed for supplement OM intake due to increasing levels of microalgae in the diet. Duodenal OM flow decreased (linear; P = 0.01) across treatments. Ruminal digestibility, expressed as a percentage of intake, and true ruminal OM disappearance did not differ (P = 0.23) with increased inclusion rates of microalgae. However OM disappearance postruminal (% of entering) decreased (P = 0.001) linearly with microalgae. Overall, N intake responded cubically (P=0.01) with increasing microalgae level in the diet. However, increasing levels of microalgae in the diet linearly decreased (P = 0.001) total N supply reaching the duodenum. A cubic affect was observed (P = 0.003) for true ruminal N digestibility. No differences (P =0.34) were observed for NDF intake, duodenal, and fecal NDF flow. Ruminal NDF digestibility (% of intake) peaked at 42% inclusion rate (quadratic; P = 0.03). In conclusion, an inclusion rate of 42% microalgae in ad libitum supplements appears to be ideal. However, the high ash content does not seem to limit intake.

Key Words: Algae, Beef cattle, Duodenal, Digestibility, Ruminal

In response to high costs and low availability of traditional fossil fuel, the use of a microalgae biofuel has become increasingly popular in past years. Microalgae factories can produce high yields per unit of cultivation (Dept. of Energy, 2010) which allow biofuels and co-products to be marketed. Beckman et al. (2013) analyzed the use of lipid extracted algae as a feedstuff for ruminant animals and found it to be a feasible inclusion to ruminant diets. However, because of the high yields of algae being cultivated some factories are unable to store and process the algae in a timely manner, leading to a product that may not contain the necessary levels of nutrients needed to be processed as a biofuel. The results from Beckman et al. (2013) prompted the potential for using the excess whole algae that falls below the set standards of biofuel grade algae as a feedstuff for ruminants. Scenedesmus sp. algae contains 45% CP and 88% TDN, 25% Ash).

Protein is one of the first limiting nutrients on rangeland, particularly during drought situations. This necessitates the need for copious amounts of supplement, which increases the labor required to maintain cows at an ideal body condition. Therefore, it would be ideal to develop feedstuffs that could be offered ad libitum to cattle on pasture who are consuming moderate to low-quality forages. Due to the relatively high ash content of Scenedesmus sp. its use as an ingredient in self-fed scenarios may serve to limit intake. We hypothesized that supplements containing Scenedesmus sp. would limit intake while digestibility between the supplements would be similar. Therefore the objectives of this experiment were to evaluate if a supplement high in Scenedesmus sp. could be used to limit intake in beef heifers fed ad libitum and to evaluate the digestibility of varying levels of microalgae in the supplement.

MATERIALS AND METHODS

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

Animals and Diets

Four crossbred beef heifers (avg BW 409 ± 18 kg) fitted with ruminal and J-style duodenal cannulas (manufactured by NMSU Range Nutrition Laboratory) were used in a 4 × 4 Latin square. Animals were housed in 3 × 2.4 m pens

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equipped with cup waters and large hay feeders with separate buckets for supplement so that intake of hay and supplement could be measured separately. Ten days before the start of the experiment heifers were offered Sudan Sorghum hay ad libitum at 5% above the previous day's intake. Heifers were provided ad libitum access to chopped (2.54 cm) Sorghum \times Sudan hay (8.8% CP and 71% NDF, OM basis) and one of four pelleted dietary treatments: 1) ad libitum access to a pelleted soybean meal based supplement (37.9% Soybean hulls, 53.0% Soybean meal, 9.1% molasses; CON); 2) ad libitum access to a pelleted supplement formulated to contain Scenedesmus sp. (microalgae) at 17% of DM (33.3% soybean hulls, 17% microalgae, 37.7% Soybean meal, 8.7% molasses, DM basis; 17%); 3) ad libitum access to a pelleted supplement formulated to contain 42% microalgae (30.3% soybean hulls, 42% Microalgae., 18.9% Soybean meal, 9.1% molasses, DM basis; 42%); 4) ad libitum access to a pelleted supplement formulated to contain 66% microalgae (24% soybean hulls, 66% microalgae, 9% molasses, DM basis; 66%). Supplements were formulated to be isonitrogenous. Daily sampling of hay and supplement started on d 1 of the study and continued through d 21 of each period. Orts were collected each day and heifers were fed at 5% above previous days' intake. Samples of individual orts for hay and supplement started on d 12 and continued through d 21 of each period. Heifers were fed twice daily at 0600 and 1800 h and given free access to trace mineralized salt (American Stockman Trace Mineralized Salt, North American Salt Co., Overland Park, KS: NaCl > 98%; Zn > 4,000 mg/kg; Fe > 1,600 mg/kg; Mn > 1,200 mg/kg; Cu 260 mg/kg; I > 100 mg/kg; Co > 40 mg/kg) Each experimental period was 21 d with a 17 d adaptation to ensure adequate adjustment of the gastrointestinal tract to the new dietary treatment and 4 d of intensive sampling. Starting on d 10 of each experimental period, gelatin boluses (size #11, Torpac Inc., Fairfield, NJ) containing 5g of TiO, were placed into the rumen twice daily at each feeding as an external marker for digesta flow (Myers et al., 2004).

Sampling

Beginning at 0400 h on d 18 of the experimental period, duodenal (200 mL) and fecal (50 mL) samples were collected every 4 h. On d 19 of the sampling period, duodenal and fecal collection times were advanced 2 h so that samples collected represent every 2 h in a 24 h period. Fecal samples were dried in a 55°C forced air oven and ground (Wiley mill, 2mm screen) and composited (equal volume) over time by heifer. Duodenal digesta samples were composited (equal volumes) by heifer for each period and immediately frozen. Duodenal digesta samples were lyophilized (VirTis Lyotroll, SP Scientific, Gardiner, NY) and ground (Wiley mill; 2mm screen). Immediately before the 0600 h feeding on d20, whole ruminal contents were removed. Whole rumen contents were collected at 3, 6, 9, 12, 15, 18, and 21 h, immediately after sample collection, whole rumen contents were placed in a blender (Hamilton Beach) with an equal volume of 0.9% NaCl (wt/vol) solution and homogenized for 1 min to dislodge particulate associated bacteria. The homogenate was then strained through eight layers of cheesecloth and immediately frozen for subsequent bacterial isolation by differential centrifugation.

Laboratory Analysis

All feed, orts, microbes, duodenal digesta, and fecal samples were analyzed for DM and ash (AOAC, 1990). Nitrogen content of feed, microbes, duodenal digesta, and feces were determined using a Leco FP-528 (St. Joseph, MI) analyzer. Neutral detergent fiber of feed, duodenal digesta, and feces were determined using an Ankom 200 fiber analyzer (Ankom Technology, Fariport, NY). Duodenal and fecal samples were analyzed for TiO_2 according to the procedures of Myers et al. (2004) using a microtiter plate reader (BioTek Instruments Inc., Winooski, VT). Microbial and duodenal samples were analyzed for purines as described by Zinn and Owens (1986).

Calculations and Statistical Analysis

Nutrient flows, digesta passage rate, and microbial efficiency were calculated as described by Scholljegerdes et al., (2004b). All data were analyzed using the MIXED procedure of (SAS 9.4) as a 4×4 Latin square experiment. The model included animal as the random variable. Autoregression order one was determined to be the most desirable covariance structure according to the Akaike's information criterion. Single-degree-of-freedom orthogonal polynomial contrasts were used to determine linear, quadratic, and cubic responses to level of supplement intake (Steel and Torrie, 1980). A significance level of 0.05 was used to separate treatment effects.

RESULTS

Intake and Digestibility of OM

Increasing levels of microalgae had no effect on forage OM intake (P = 0.13) however, a cubic effect was observed for supplement OM intake (P = 0.01) with increasing levels of microalgae in the diet. Total OM intake tended to respond cubically (P = 0.08) to increasing levels of microalgae in the diet (Table 2). Furthermore, the cubic response in total OM intake could be influenced by the cubic response to the supplement OM intake. Duodenal OM flow decreased (linear; P = 0.01) with increasing levels of microalgae to the diet. Similarly microbial OM flow to the duodenum decreased (P

Table 1. Chemical composition of feedstuffs fed to beef heifers¹

Item	Hay	Con	17%	42%	66%
DM	94	92	91	91	90
OM, % of DM	86	92	89	86	83
CP, % of OM	8.8	29.8	32.2	30.9	31.3
NDF, % of OM	71	29.5	30.5	30.5	31
Ash, % of DM	13.6	7.7	10.7	14.2	17.4

¹Samples for nutrient analysis were taken on d 12 through d 19 of each period.

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Item	Con	17%	42%	66%	SEM^2	Linear	Quad	Cubic
OM Intake, g/d								
Forage	2931	3785	2856	4458	673	0.23	0.58	0.13
Supplement	4195	2896	5542	2714	534	0.45	0.16	0.006
Total	7302	6762	8173	7140	568	0.70	0.66	0.08
OM flow, g/d								
Duodenal	2678	2169	1863	1940	180	0.01	0.12	0.81
Microbial	889	715	617	651	59	0.01	0.09	0.83
Fecal	1312	1150	1213	1248	87	0.64	0.14	0.37
OM disappearance								
True ruminal. % of intake ³	66.6	67.2	67.4	66.1	0.83	0.72	0.23	0.75
Postruminal, % of entering	51.1	46.4	34.7	35.2	2.0	0.001	0.25	0.09
Total tract, % of intake	81.6	83.0	85.0	82.1	0.95	0.36	0.06	0.16
N intake, g/d	305	234	374	231	30.0	0.55	0.25	0.01
Duodenal N flow. 2/d								
Total	172	137	107	105	12.4	0.0007	0.09	0.47
Microbial	94	73	61	61	5.87	0.002	0.07	0.82
Non-microbial N	76	99	49	41	10.8	0.005	0.86	0.53
Fecal N flow, g/d	46	54	51	48	5.45	0.93	0.21	0.49
N digestibility								
True ruminal, % of intake								
	74	67.6	85.8	79.9	4.67	0.02	0.93	0.003
Lower tract, % of duodenal flow								
	71.9	62	52.7	54.2	3.8	0.01	0.18	0.57
Total tract, % of intake	84.4	73.6	85.7	76.4	3.8	0.50	0.83	0.02
NDF intake g/d	3433	3613	3634	4010	415	0.34	0.81	0.74
Duodenal NDF flow, g/d	877	670	609	673	83.5	0.12	0.15	0.96
Fecal NDF flow, g/d	839	711	772	795	71.5	0.79	0.22	0.46
NDF digestibility								
Ruminal, % of intake	74	81.2	84	83.2	1.4	0.002	0.03	0.70
Total tract, % of intake	74	80	79.1	80	2.1	0.10	0.22	0.28

Table 2. Influence of increasing fat levels on OM intake, flow, and digestibility in beef heifers consuming native grass hay

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= 0.01) linearly. When ruminal digestibility is presented as a percentage of intake, no differences (P = 0.23) were observed for true ruminal OM disappearance across increasing microalgae levels in the diet. However OM disappearance postruminal (% of entering) decreased (linear; P = 0.001) as microalgae level increased in the diet. There was a tendency (quadratic; P = 0.06) for total tract OM disappearance (% of intake) to increase to the 42% inclusion rate then decrease at 66%.

Intake and Digestibility of N

Overall, N intake responded cubically (P = 0.01) with increasing microalgae level in the diet (Table 2). Scholljegerdes et al. (2008) showed a linear increase of N

intake with additional free choice flaxseed. The cubic effect observed may be attributed to the cubic response in supplement intake. However, increasing levels of microalgae in the diet linearly decreased (P = 0.001) total N supply reaching the duodenum. Duodenal supply of microbial N decreased (P = 0.002) linearly as microalgae level in the diet increased. Similarly duodenal non-microbial N flow decreased (P = 0.003) for true ruminal N digestibility. While lower tract N digestibility decreased (linear, P = 0.01) across treatments. Total tract N digestibility followed a similar pattern as true ruminal N digestibility with a cubic response (P = 0.02) inclusion of microalgae.

Intake and Digestibility of NDF

No differences were observed (P = 0.34) for NDF intake or duodenal and fecal NDF flow (Table 2). This agrees with Scholljegerdes et al. (2008) that observed no differences for NDF intake or duodenal and fecal NDF flow. However, ruminal NDF digestibility (% of intake) had a quadratic response (P = 0.03) and total tract NDF digestibility tended to increase (P = 0.10) linearly across treatments.

DISCUSSION

To our knowledge we are the first to report the investigation of ad libitum access to supplements that high in protein (32% CP) and ash (x% ash), particularly from the microalgae Scenedesmus sp. Supplements used on range settings are generally high in crude protein. Mathis et al. (2000) supplemented RUP and found that bermudagrass intake did not differ. The forage fed by Mathis et al. (2000) was similar in CP (8.2%) to that fed in the current experiment (8.8%). Conversely, increasing the level of supplemental soybean meal did increase forage intake when low-quality forage (4% CP) was provided ad libitum (Guthrie and Wagner, 1988). Supplement intake exhibited a cubic response as inclusion rate of microalgae increased. Specifically, supplement intake decreased with the inclusion of 17% microalgae and increased at 42% then decreased at 66%. This response was not noted in ruminal OM digestibility, in which no differences were observed. Therefore, it is difficult to describe what biological phenomena are taking place to cause this cubic response. The lack of difference in forage intake is supported by Moore et al. (1999), in which voluntary forage intake did not differ with supplemental protein when forage CP was above 7%.

True ruminal OM digestibility did not differ across treatments. This True ruminal N digestibility exhibited a cubic response, which was largely due to the moderate decrease from Con to 17%, with a marked increase for the 42% treatment followed by a decrease observed for the 66% treatment. The variation amongst supplement responses to ruminal digestibility may be due to changes in TDN:CP ratio's in the rumen (Moore et al., 1999). Postruminal disappearance of OM and N decreased linearly. This reduction in digestibility, which increased linearly and not overall OM, which did not differ. Total tract OM and N digestibility tended to respond quadratically and cubically, respectively. These results are due in part to the similar responses in intake.

IMPLICATIONS

The results presented here indicate a potential outlet for whole algae to be used as a feedstuff for ruminant animals if it falls below the standard s of biofuel grade algae. However as one increases the amount of algae present in a feedstuff, the amount of microalgae will also increase which can potentially hinder the amount of nutrients reaching the small intestine. The results of this study reveal a number of cubic responses that could be attributed to the animals adjusting to the feed or the high level of microalgae may decrease intake.

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Effect of delaying time AI based on ESTROTECT[™] patch status on pregnancy rates of nursing beef cows

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ABSTRACT: The objective of this study was to evaluate the use of an EstrotectTM patch status at 58 h post controlled internal drug release (CIDR) removal and PG injection when using the CO-Synch + CIDR protocol in lactating beef cows to evaluate pregnancy rates to timed AI (TAI) at either 58 or 76 h. Bos taurus cows (n = 997) across 6 locations were administered a 7-d CO-Synch + CIDR synchronization The experiment involved a 2×2 factorial protocol. arrangement of treatments; factors were patch status at 58 h post PG injection (activated or unactivated) and time interval from PG to TAI (58 or 76 h). Patch status was characterized at 58 h into 4 scores: 1 = 0% activated, 2 = 50% activated, 3 = 100% activated, and 4 = missing. Cows with a patch status of 3 were considered to be activated and patch status of a 1 or 2 were considered to be unactivated. Cows with missing patches were removed from the overall factorial analysis. There was no treatment \times location interaction (P = 0.96), so all data were pooled across locations. There was a tendency (P = 0.07) for an interaction between the interval from PG to TAI and patch status for pregnancy rate. When main effects were analyzed, pregnancy rate was greater (P < 0.01) for cows with activated patches at 58 h post PG compared to those with unactivated patches. There was no difference (P =0.99) for pregnancy rate when comparing the 58 h (59.6%) vs. 76 h PG to TAI interval (59.7%). Pregnancy rates for cows with a patch status of 3 at 58 h post PG were greater (P < 0.05) than those cows with patch statuses of 1 or 4 and tended (P = 0.09) to be greater than cows with a patch status of 2. Although it was not significant (P = 0.13), there was a 7.4 percentage point increase in pregnancy rates for cows with unactivated patches that received TAI at 76 h vs. 58 h. Results demonstrate that cows with an activated patch at 58 h had higher pregnancy rates than cows with unactivated patches. The interaction between patch status and PG to TAI interval (P = 0.07) could be of importance for increasing precise timing of AI in mature beef cows.

Key Words: AI, beef cows, delayed insemination, estrus detection aid

INTRODUCTION

Estrous synchronization and AI are some of the most important and widely applicable reproductive technologies available for beef cattle producers (Seidel, 1995). Researchers are developing estrous synchronization protocols using 2 key factors to help encourage implementation: 1) minimize frequency of handling, and 2) eliminate detection of estrus by employing TAI (Lamb et al., 2006). Difficulty associated with detection of estrus is one of the primary reasons that many cattle producers do not use AI, but development of estrus detection patches could help eliminate this obstacle to implementation (NAHMS, 1994). The activated patches would allow cows to be classified at TAI as having exhibited estrus. Those females with an activated patch would receive TAI while the remaining cows would be induced to ovulate via an injection of exogenous GnRH and subsequent TAI at a later time (18-20 h). Semen in the female reproductive tract has a lifespan of 24 h (Trimberger, 1948), which can result in not having viable spermatozoa for cows that did not exhibit estrus. Sperm transport to the site of fertilization in the oviduct requires a minimum of 4 to 6 h following insemination in the cow (Hunter and Wilmut, 1983). Therefore, the optimal time for AI is 6 to 16 h after the onset of estrus (Dransfield et al., 1998) and 6 h after the second GnRH injection of an Ovsynch protocol (Pursley et al., 1998). Therefore, we hypothesized that cows with activated patches will have greater pregnancy rates at 58 vs. 76 h post PG, and cows with unactivated patches will have greater pregnancy rates at the 76 vs. 58 h.

MATERIALS AND METHODS

Postpartum beef cows (n = 997) in 6 herds and across 3 states were enrolled in this study. All cows had a CIDR (Zoetis, Florham Park, NJ) inserted intravaginally and were given 100 µg of GnRH (Factrel, Zoetis) on d 0. On d 7 all cows had CIDR removal and were given 25 mg of PG (Lutalyse, Zoetis) and Estrotect[™] patches (Estrotect[™], Spring Valley, WI) applied to their tail head. Fifty-eight hours after the PG injection, all cows were given 100 µg of GnRH (Factrel, Zoetis), and approximately half of the cows (58 h = 510 cows, 76 h = 487 cows) were inseminated. At 58 h post PG, all cows had their EstrotectTM patches characterized into 4 scores: 1 = 0% activated, 2 = 50% activated, 3 = 100%activated, and 4 = missing. Cows with a patch status of 1 or 2 were considered to be unactivated and a patch status of 3 was considered to be activated. Cows with missing patches were removed from the overall factorial analysis. At 76 h post PG, the remaining cows were inseminated. Cows were randomly designated to the 58 or 76 h group by ear tag number or randomly selected by chute order. At 58 h, all calves were removed from their dams and held separately until after cows were inseminated. Cows inseminated at 76 h were left separated from their calves beginning at 58 h through insemination at 76 h.

Statistical Analysis

Data were analyzed as a 2 \times 2 factorial using the GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC) to produce a general linear mixed model including the fixed variables of BCS, post-partum interval, patch status, PG to TAI interval, and patch status \times PG to TAI interval. Location was set as a random variable in the model. There was no treatment \times location interaction (P > 0.05), so data were pooled across locations. A contrast statement was used in the model to examine differences between groups of means within the factorial. Means were compared and separated using the LSMeans option in SAS.

RESULTS AND DISCUSSION

Body Condition Score and Post-Partum Interval

The number of cows, d postpartum, and mean BCS at the time of CIDR insertion on d 0 are presented in Table 1. On average, cows were in adequate BCS across all locations. Cows from locations 2 and 3 had greater (P < 0.05) BCS than cows at other locations. Post-partum intervals were not different (P > 0.05) when comparing locations 2 and 5. However, all other locations differed from each other for post-partum interval (P < 0.05).

Patch status

The pregnancy rate for cows with a patch status of 3 (67.3%) was greater (P < 0.05) than cows with a patch status of 1 or 4 (50.0 and 52.5%), respectively. There was a tendency (P = 0.09) for cows with a patch status of 3 to have higher pregnancy rates (67.3%) than cows with a patch status of 2 (56.6%). This result is similar to data reported by Bridges et

Table 1. Average BCS and post-partum intervals among locations and overall

Location	n	BCS^1	SE	PPI ²	SE
1	263	5.1 ^a	0.03	86.2 ^b	0.87
2	93	5.3 ^b	0.07	74.5 ^a	1.43
3	180	5.5°	0.04	116.6 ^e	0.68
4	128	5.1 ^a	0.05	93.3°	1.88
5	155	5.1 ^a	0.03	73.4 ^a	0.72
6	178			97.3 ^d	1.31
Overall	997	5.2	0.02	91.3	0.79

¹BCS on 1 to 9 scale (Wagner et al., 1988)

²PPI = post-partum interval

^{a-e} Among locations means within a column lacking common superscripts differ (P < 0.05).

al. (2012), in which cows that were assumed to have shown estrus activity, via rubbing off tail paint before or at TAI had greater pregnancy rates than those that were not rubbed. With the development of the CO-Synch + CIDR protocol and control of ovulation, it is possible for some cows that have not shown estrus to conceive. Stevenson et al. (2000) noted that a second injection of GnRH reduced estrus expression from 79.5 to 13.0%. This is because the LH surge triggered by GnRH will suppress estrogen levels of the follicle and the female will not exhibit estrus, leading to ovulation of the dominant follicle (Twagiramungu et al., 1995).

Delayed Fixed-Time AI Pregnancy Rates

There tended (P = 0.07) to be an interaction between patch status and PG to TAI interval. Since the interaction was not significant main effects were evaluated individually. Cows with activated patches had greater (P < 0.01) pregnancy rates (68.0%) compared to those whose patch was unactivated (50.7%). This is similar to results reported by Busch et al. (2008) where cows that were observed in estrus had higher pregnancy rates than cows who did not show estrus. In the current study, there was no effect on pregnancy rates between 58 (59.6%) and 76 h (59.7%) PG to timed AI interval (P > 0.05). Previous research by Geary and Whittier (1998) compared CO-Synch to Ovsynch and by using the Ovsynch protocol and waiting an additional 24 h, pregnancy rates were increased by 8 percentage points (Geary and Whittier, 1998). However, this research was done prior to inclusion of CIDR inserts in either of these protocols which may explain the difference between this study and the current study. This differs from this research that suggest 58 or 76 h interval has no effect on pregnancy rates. When comparing cows with unactivated patches and given TAI at 58 h to cows with unactivated patches and given TAI at 76 h there was only a numerical advantage, 47.0 vs. 54.4%, respectively (P = 0.13). There was no difference when comparing cows with

Fable 2.	Distribution of	of Estrotect	[™] patch	status at
58 h pos	st prostagland	in injection	and pre	gnancy

	rate among pa	ten status	
Patch status ²	Percent of	Pregnancy rate	SE
Status	0043	(70)	
1	49.1	50.0 ^a	2.3
2	7.7	56.6 ^{ab}	5.7
3	35.2	67.3 ^b	2.5
4	8.1	52.5ª	5.6

 $^{1}n = 997$

 $^{2}1 = 0\%$ activated , 2 = 50% activated, 3 = 100% activated, 4 = missing

³ Estrotect[™], Spring Valley, WI

^{a-b}Means within a column lacking common superscripts differ (P < 0.05).

activated patches and given TAI at 58 h to cows with activated patches and given TAI at 76 h, (71.0 vs 64.7%); (P = 0.26). From data collected using the factorial in the current study an improved strategy was comprised of evaluating patch status at 58 h and insemination of cows with activated patches at 58

h and inseminating cows with unactivated patches at 76 h. Using a contrast statement this strategy was compared to the overall mean pregnancy rate. When comparing this strategy, pregnancy rates tended to be higher (P = 0.07) than the overall mean (64.2 vs. 56.9). When comparing this strategy

			Patch status at 58 h ² (%)			
Location	Pregnancy rate (%)	SE	1	2	3	4
1	59.7 ^b	3.0	58.9 ^e	6.1 ^{ab}	32.7 ^{ad}	2.3ª
2	60.2 ^b	5.1	66.7 ^e	2.2^{a}	22.6 ^a	8.6 ^b
3	59.4 ^b	3.7	42.8 ^{bc}	5.0 ^a	51.1°	1.1^{a}
4	41.7 ^a	4.4	55.1 ^{de}	11.8^{bc}	32.3 ^{ad}	0.8^{a}
5	63.2 ^b	3.9	29.0 ^a	12.9 ^{be}	40.0^{ac}	18.1°
6	53.9 ^b	3.7	45.1 ^{bcd}	8.0^{ac}	26.9 ^d	20.0 ^c
Overall	56.9	1.6	49.1	7.7	35.2	8.1

 Table 3. Pregnancy rates to timed AI and percentage within each patch status by location and overall

 $^{1}n = 997$

 $^{2}1 = 0\%$ activated, 2 = 50% activated, 3 = 100% activated, 4 = missing

^{a-e} Among locations means within a column lacking common superscripts differ (P < 0.05).

Table 4. Effect of time interval (58 vs. 76 h) from PG to timed AI and Estrotect[™] patch status at 58 h post PG on pregnancy rate by location ¹²

	58 h ³	76 h ³	58 h ³	76 h ³		P values	
Location	Activated ⁴	Activated ⁴	Unactivated ⁴	Unactivated ⁴	Interaction	Interval	Patch status
1	76.3	67.5	49.3	54.8	0.26	0.71	< 0.01
2	77.7	100.0	50.0	51.7	0.98	0.98	0.97
3	68.4	65.2	41.5	58.5	0.21	0.41	0.03
4	58.4	37.7	35.7	43.8	0.21	0.60	0.46
5	73.5	62.0	64.4	64.6	0.49	0.51	0.69
6	64.1	54.5	56.5	49.7	0.86	0.39	0.51
Overall	71.0 ^e	64.8 ^{bc}	47.0 ^a	54.4 ^b	0.07	0.99	< 0.01

 $^{1}n = 912$

² EstrotectTM, Spring Valley, WI

³ Prostaglandin to timed AI interval.

⁴ Activated = patch status of a 3 (100% activated); unactivated = patch status of a 1 or 2 (0% activated or 50% activated, respectively) evaluated at 58 h post PG.

⁵ Least Squares Means are reported in this table.

⁶For this analysis cows with a patch status of a 4 were removed.

⁷Location 2 and 6 used different models to come up with their Least Squares Means with location.

Consisted of postpartum interval, patch status, interval, and patch status x interval.

⁸There was no treatment x location interaction (P = 0.96).

^{a-c} LSMeans within a column lacking common superscripts differ (P < 0.05).

to the 58 h PG to TAI main effect there was no difference in pregnancy rate for the improved strategy (64.2 vs. 59.6); (P = 0.13). Similarly there was no difference when the improved strategy was compared to the 76 h PG to TAI interval (64.2 vs 59.7); (P = 0.26). Pregnancy rates for location 4 were lower than all other locations (P < 0.05). However, all other locations were similar (P > 0.05).

The Beef Reproductive Task Force recommends that the standard 7-d CO-Synch + CIDR TAI protocol have a 60 to 66 h interval from PG to TAI. This is supported by data from Wilson et al. (2010) in which the average interval from PG to estrus is 64.8 h using the 7 – d Select Synch plus CIDR protocol. For the current study, an 18 h delay was used; therefore, CIDR's were pulled early on d 7 so TAI could occur on the afternoon of d 9 and morning of d 10 in daylight hours. Slight variation from the standard 60 to 66 h timing interval was established to ensure that breeding was done in daylight hours and sufficient time was allowed to process all cows.

In conclusion, results suggest the interaction between patch status and PG to TAI interval (P = 0.07) could be of economic importance for increased overall TAI pregnancy rates. Moreover, TAI pregnancy rates increased in cows with activated patches. There was no difference in pregnancy rates when comparing the 58 or 76 h interval.

IMPLICATIONS

Through the evaluation of patch status at 58 h post PG and inseminating cows with activated patches at 58 h and cows with unactivated patches at 76 h, this delay has a tendency to yield greater pregnancy rates than the overall mean. By using this delayed TAI protocol, only a subset of cows with unactivated patches would need to be inseminated at 76 h. By sorting calves from cows at 58 h and leaving them sorted until after insemination, calf sorting will be minimized. In order to stay true to this protocol's timeline, CIDR's may be pulled from cows early in the morning on d 7 to ensure that there is adequate daylight during insemination time at 58 h. Cows may need to be gathered the night of d 6 to ensure that all CIDRs will be pulled in a timely manner.

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Economic analysis of cow longevity

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ABSTRACT: Longevity and the length of time of peak cow performance contribute positively to increased cow-calf herd profitability. Reproduction records, calf weights, and cow removal records from a longevity project that evaluated cows produced by AI matings of Angus, Gray Brahman, Red Brahman, Indu-Brazil, Gir, and Nellore bulls to Hereford cows from 1985 to 2005 were utilized to determine the economic impact of increasing the productive lifespan of a beef cow, and the economic gains from a longer peak productive life. Net present value was estimated for cows using a stochastic simulation model. Average NPV was the highest for the Nellore crossbreds (\$2,338; 95% confidence interval (CI): \$2025.08, \$2593.10) and lowest for the Angus crossbreds (\$245; 95% CI: -\$7.48, \$491.21). All the breed groups in the study were found to have an average NPV that was significantly different from that of the base breed group (P < .001). The value of inclusion of an additional year of peak performance ranged from \$118 (95% CI: \$24.24, \$203.48) per cow for the Angus crossbreds to \$244 (95% CI: \$110.50, \$340.39) per cow for Indu-Brazil sired cows. The economic impact of increasing longevity or increasing the number of peak production years was positive and large. When age at culling was compared, it was found that Nellore and Gir crossbreds had means that were significantly different (P < .05) from the base, Angus crossbreds. All other breeds were found to not have means that were significantly different (P > .05).

Keywords: cow, longevity, net present value

INTRODUCTION

Longevity of cows can be defined as the length of time that a cow is productive. Longevity in beef production is based around avoidance of removal from the herd, which would primarily occur because of reproductive failure or the production of poor quality calves. Shafer (2006) stated that producers have long known that cow longevity is a key component of profitability puzzle.

The value of longevity is affected by the opposite value of removal and the cost of replacement. Cows achieve peak performance from 8 to 10 yr of age (Bourdon and Brinks, 1987). The optimal age of removal has been investigated from a production perspective, but the economic impacts of increasing the productive life of that beef cow have not been adequately detailed (Rogers, 1972; Tronstad et al., 1993; Ibendahl et al. 2004). The objective of this research was to determine the economic impact of increasing the productive lifespan of a beef cow and the economic gains from a longer peak productive life.

MATERIALS AND METHODS

The data used in this study was collected from the Texas A&M AgriLife Research station at McGregor. The 116 cows with records in this study were born from 1982 to 1985, and were F₁ crosses. The dams of these F₁ cows were purebred Hereford cows. Angus, Gray Brahman, Gir, Indu-Brazil, Nellore, and Red Brahman bulls were sires of the cows in the study. Paschal et al. (1991; 1995) reported the birth and weaning characteristics of these cows and their half-siblings, and the stocker performance, feeder performance, and the carcass characteristics of the steers that were siblings to the F₁ cows used in this study. Riley et al. (2001a, b) compared the differences in reproduction, maternal ability, size traits, udder characteristics, loss of incisors with aging, longevity, and lifetime productivity for the cows of these breed types. Cows were exposed to bulls annually in 78-d breeding seasons in multiple-sire pastures. Cows were removed from the project after 2 failures to wean calves (from annual exposure to bulls) through 12 yr of age. Subsequently cows were removed after a single failure to wean a calf. Cows were removed at any time for severe udder problems, structural unsoundness, and health conditions. A total of 1,278 calves were born to these cows from 1985 through 2005.

Net present value (NPV) is defined as the present value of the revenue minus costs over the investment period. NPV is commonly utilized to analyze the profitability of an investment. In this case, the investment is the cow. Revenues are generated from the calves sold and the salvage value of the cow.

$$NPV = \sum_{i=1}^{t} \frac{REV - COST}{(1+i)^t}$$

Where:

NPV = net present value REV = cash inflows COST = cash outflows i = discount rate t = (cow age in years - 2)

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The NPV was calculated on a per cow basis. The effect of increased longevity was evaluated in terms of the difference in NPV. Stochastic simulation techniques, using Simetar (Richardson et al., 2008) were used to account for variability in steer and heifer weaning weight and the weaning rate based on the variability observed in the cow data. Simulation provides the opportunity to make probabilistic estimates of alternative strategies based on the estimated distributions of economic returns.

It was assumed that during the cow would not incur the full costs associated with remaining in the herd for an additional year during her final year. Therefore, the cost of maintaining the cow per year was not subtracted from the final year that she was alive in the herd. Average annual cow costs were estimated to be \$600 at the end of the collection of data.

The average weaning weight for steers and heifers were taken independent of each other for each year that a cow was part of the project. Regression analysis was employed to predict the annual weights of the steers and heifers weaned by cows. After first calving as 2.5 year olds the cows were subsequently bred to calve at 3.5 years and so on, until the end of the study.

The model is a basic Net Present Value (NPV) model. It takes into account revenue and the cost associated with each cow in the herd. Revenue was determined by multiplying the probability a cow would wean a steer with the stochastic weight of a steer and the stochastic price of a steer for that particular year. This value was then added to the dollar amount generated by multiplying the probability of the cow having a heifer calf by the heifer price and stochastic heifer weight. A cost of \$600 for maintenance was deducted every year that a cow was in the herd. The profit (loss) dollar amount was then discounted to its present value. The net present value for each year was summed up for each cow individually; this yielded the NPV for each.

For the year that the cow was removed, revenue was increased, if applicable, by multiplying her final weight with the stochastic price for a cull cow. If the cow died or was culled for reasons that would make her unfit for sale and slaughter, her value at culling was not included in the model.

To determine the effect of one additional year of peak productivity, it was decided that an additional year where the average NPV of the breed group was highest would be added again. This meant that if the average peak year for Angus cows was at age 6, then an additional year, using data from age 6 was added and discounted to the overall NPV of all Angus sired cows.

Changes in dollar value across project duration were also modeled, as well as the differences in weaning rate and weaning weight across the different breeds utilized in the study. Weaning weights were correlated to the probability that the cow had either a bull or heifer calf during that particular year. Price data was stochastically simulated. The weaning rates for cows were modeled based on sire breed and cow age.

RESULTS AND DISCUSSION

Weaning weights declined with age past peak production years. Net Present Value became negative for each cow at some age when costs exceeded the revenue from the calf produced at that age. The age at which annual NPV became negative ranged from 11 for the Indu-Brazil crosses to 15 yr for the Nellore crosses (Table 1). Net Present Value for the Angus and Gray Brahman crossbred became negative on average at 12 and 13 yr of age, respectively. Average NPV was the highest for the Nellore crossbreds (\$2,338) and lowest for the Angus crossbreds (\$245, Table 1). The NPV was affected strongly by the number of failures to wean a calf, the number of years calves were weaned, and the weights of the weaned calves. Nellore crossbreds had the longest productive life and weaning rate (Riley et al., 2001a, b). When compared to the base breed, Angus breed group, all of the other breed groups in the study had average NPVs that were significantly different (P < .05).

The value of inclusion of an additional year of peak performance ranged from \$118 per cow for the Angus crossbreds to \$244 per cow for Indu-Brazil sired cows (Table 2). As expected, an additional peak year of production generated a positive economic return and increased cow value.

Cow age at culling was also evaluated (Table 3). Angus, Gray Brahman, Indu-Brazil, and Red Brahman all had similar average age at culling, they were culled at an average age of 12.8, 12.95, 12.36, and 13.14 years, respectively. While, Nellore F_1 and Gir F_1 cows had an average age at culling that was approximately 3 years longer than the other breeds. For the purposes of this study Angus F_1 crossbreeds were used as the base when testing for p-values. A p-value was assumed to be statistically significant if it was less than .05. When compared to the age at culling for Angus F_1 crossbreeds, only Gir F_1 cows and Nellore F_1 cows had statistically significant differences in their age at culling (P < .05). The other three breeds analyzed in the study did not have an age at culling that was significantly different (P > .05) to the base breed.

IMPLICATIONS

Greater longevity and longer peak productivity generate increased value. Additional lines of inquiry include determination of optimal ages of removal for each breed type, and identification of economically appropriate removal criteria for individual cows. Combining selection procedures with economic decision processes could strongly impact profitability.

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Sire of dam breed	Ν	Average age (yr) with 1st negative NPV	Total NPV	Average NPV per cow
Angus	15	12	\$ 3,685.65	\$ 245.71
			(\$571.73, \$7420.68)	(-\$7.48, \$491.21)
Gray Brahman	21	13	\$ 20,642.83	\$ 982.99
			(\$16,513.65, \$24,710.82)	(\$791.84, \$1172.29)
Gir	15	15	\$ 27,937.92	\$ 1,862.53
			(\$23,720.85, \$32,127.98)	(\$1564.10, \$2152.17)
Indu-Brazil	19	11	\$ 25,728.16	\$ 1,354.11
			(\$19,285.70, \$30670.38)	(\$990.21, \$1610.38)
Nellore	25	15	\$ 58,457.66	\$ 2,338.31
			(\$50,656.24, \$64,830.53)	(\$2025.08, \$2593.10)
Red Brahman	21	13	\$ 22,841.03	\$ 1,087.67
			(\$21,100.08, \$24,580.54)	(\$991.11, \$1177.13)

Table 1. Average net present value (NPV) of each sire of breed group (95% CI)^{1, 2}

¹CI = confidence interval

 ^{2}N = number of cows with records in each group.

Sire of dam breed	N	Total original	NPV +1 peak productive	Average NPV per	Change in average NPV
		NPV	year	cow	per cow
Angus	15	\$ 5,278.49	\$ 5,455.28	\$ 363.69	\$ 117.98
			(1,588.08, 9,667.35)	(62.78, 653.72)	(24.24, 203.48)
Gray Brahman	21	\$ 19,980.45	\$ 21,895.73	\$ 1,042.65	\$ 59.66
-			(17,308.25, 26,803.08)	(836.22, 1,231.74)	(-0.10, 119.14)
Gir	15	\$ 27,255.02	\$ 31,233.82	\$ 2,082.25	\$ 219.72
			(26,192.46, 36,122.51)	(1,738.17, 2,390.60)	(123.25, 294.30)
Indu-Brazil	19	\$ 26,729.52	\$ 30,830.13	\$ 1,598.95	\$ 244.84
			(22,998.71, 36,171.68)	(1,207.95, 1,915.61)	(110.50, 340.39)
Nellore	25	\$ 58,664.73	\$ 64,453.67	\$ 2,578.15	\$ 239.84
			(55,944.13, 71,684.01)	(2,242.88, 2,848.43)	(142.35, 311.45)
Red Brahman	21	\$ 22,291.73	\$ 26,663.99	\$ 1,269.71	\$ 182.04
			(24,788,37, 28,743,65)	(1.162.95, 1.374.80)	(145.99, 219.23)

Table 2. Average net present value (NPV) of breed groups after inclusion of one additional peak year (95% CI)^{1, 2, 3}

¹The additional year was generated by replicating the year where the average NPV of the breed group was highest.

²CI = confidence interval.

³N = number of cows with records in each group.

Sire of dam breed	Ν	Average age at culling	Minimum cow age	Maximum cow age
Angus	15	12.80	3	17
Gray Brahman	21	12.95 ^b	3	18
Gir	15	15.13 ^a	6	19
Indu-Brazil	19	12.37 ^b	6	17
Nellore	25	15.20 ^a	4	21
Red Brahman	21	13.14 ^b	3	18

Table 3. Comparison of age at culling of the breed groups

^a The mean is statistically different from the base (P < .05) ^b The mean is not statistically different from the base (P > .05)

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Evaluation of sampling error in the measurement of body weight gain and dry matter intake for the calculation of residual feed intake in growing purebred Charolais cattle

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INTRODUCTION

ABSTRACT: The objective of this study was to determine relative contributions of sampling errors of measurements used to evaluate residual feed intake (RFI) and identify the possibilities of measuring body weight gain over a greater time-span than the period used to estimate feed intake. Weaning weight (WW), ADG, and individual DMI, were recorded on 970 growing, Charolais bulls (n = 519) and heifers (n = 451) using a GrowSafe (GrowSafe, Airdrie, Alberta, Canada) system at the Simplot Livestock Company's Grand View Feedlot (Grand View, ID). Averages of individual DMI were calculated in 10-d increments and compared to the overall DMI, to identify the magnitude of the errors associated with measuring DMI. Incremental measurements were used to calculate RFI; computed from linear regressions of DMI on ADG and midtest BW0.75 (MMWT). Two alternative measures of ADG were considered; either from the standard 70-d test period for which DMI was recorded or from weaning to final out-weight. Means (SD) for DMI, ADG CALC, ADG WW, RFI CALC and RFI WW were 8.59 (SD = 1.44) kg.d-1, 1.36 (SD = 0.37) kg.d-1, 1.24(SD = 0.25) kg.d-1, 0.00 (SD = 0.650) kg and 0.00 (SD = 0.650)0.647) kg, respectively. Average DMI was highly correlated with reduced period measurements, with 10 d showing the lowest correlation (0.85, P < 0.01) and 60 d the greatest correlation (0.99, P < 0.01). Although all were significant, variation appeared to stabilize at 30 d (0.95, P < 0.01). The ADG CALC and ADG WW were highly correlated (0.61, P < 0.01), as was RFI CALC (RFI calculated using ADG Calc) with RFI WW (RFI calculated with ADG WW) (0.96, P < 0.01). DMI estimates using shorter DMI estimate periods were included in the model with the weaning weight gain measurements, all values were highly correlated with RFI CALC. The RFI 10 WW (RFI calculated with ADG WW and 0-10 DMI) showed the lowest correlation with RFI CALC (0.71, P<0.01) and RFI 60 WW (RFI calculated with ADG WW and 0-60 DMI) showed the greatest correlation (0.95, P < 0.01). These analyses indicate that reduction in the standard 70-d period to estimate RFI is most affected by loss of confidence in estimating ADG; whereas, loss in confidence in measuring DMI using only 40 d of DMI measurements is only marginally affected. We conclude, that while 70 d is required to accurately estimate ADG, a shorter period, possibly as few as 40 d is needed to accurately estimate DMI for a reliable calculation of RFI.

Key words: residual feed intake, growing bulls

By year 2050 the world will have to produce one hundred percent more food to feed the global population (United Nations, 2009). The majority of this production will have to be generated by new technologies or improved efficiencies due to the finite amount of natural resources at our disposal (Godfray, et. al., 2010). Beef production has flourished by increasing the efficiency and gain of the animals, in 2007, compared to 1977, the US generated 12% more beef with 88% of the cattle inventory (Capper, 2011). The increases in efficiencies have come from improvements in management, technologies and genetic merit of the national beef herd. Newer technologies have made it possible to measure feed intake on individual beef animals on a large scale. Residual feed intake is a calculation for measuring feed efficiency that is unrelated to weight or growth (Koch et. al., 1963; Archer et. al., 1999; Arthur et.al., 2001; Wang et. al., 2006). This has allowed the industry to select for animal efficiency more easily. The investment that must be made in the equipment is not trivial. Most producers cannot justify the investment needed for the equipment and must rely on centralized testing facilities. The current standard test for measuring feed intake and body weight gain is seventy days (Archer, et. al., 1997). By changing the current feed intake and ADG measuring paradigm it would be possible to increase the number of animals that are measured through testing facilities. Ultimately reducing the cost of the test and increasing producer adoption.

MATERIALS AND METHODS

Animals and Management

Data were collected using feed intake measuring equipment (GrowSafe Systems LTD., Airdrie, Alberta) at the Simplot Livestock Company's Grand View Feedyard (Grand View, ID). Nine hundred and seventy purebred Charolais bulls (n=519) and heifers (n=451) were tested at the Grand View facility from 2011 through 2013 all animals belonging to the Simplot Precision Genetics herd. The animals were grouped into 18 cohorts based upon gender, breed, test peers and to control for pen effect where present. Trial durations ranged from 66 days to 100 days. These durations were affected by the demand upon the facility and failure days of the system. Complete descriptions of the facility and feeding management can be found in Kayser and Hill (2013).

Average Daily Gain Measurements

Two different calculations were used to quantify the ADG of the animals. The ADG_Calc was calculated as the difference between the average start and finish weights measured on consecutive days divided by the study duration. The ADG_WW was calculated as the difference between the last out weight and the weaning weight divided by n days between the two weights.

Feed Intake Measurements

Feed intakes were recorded with a GrowSafe 4000E feed intake system. GrowSafe data acquisition and analysis software was used to convert data into readable formats for subsequent analysis. For data integrity and quality control purposes, daily assigned feed disappearance (AFD) for each feeding unit was reconciled against the total daily feed delivered to each bunk versus the sum of the daily consumption for each bull. Data were considered valid for analysis for all days on which AFD and feed delivered values were > 95 % agreement. The percent of valid days on average were 91% for the Grand View facility. Data collected on a day for which this criterion was not met were excluded from all analyses. DMI was calculated as the average of the dry matter consumed for all valid days. Averages of DMI were also calculated on 10 day increments from 10 to 60 days. These values were compared against each other as well as used in the RFI model to identify the necessary amount of days needed to measure DMI.

RFI Computations and Statistical Analysis

Statistical analyses were conducted with the SAS system (Version 9.3, SAS Inst., Cary, NC). The RFI values were calculated as the difference between actual and predicted feed intake by regressing DMI on mid-test BW0.75 and ADG (Koch et al., 1963, Archer et al., 1997). Residual feed intake was determined within cohort (breed, year, gender and location); pen effects were controlled for when needed. The regression analysis used to determine RFI was conducted using the GLM procedure in SAS. RFI values were calculated using the two different aforementioned values of ADG along with the different measures of DMI. Partial correlation coefficients were calculated using the Proc GLM procedure in SAS and were controlled for by cohort. All means were calculated using Proc means and were calculated for each breed and gender.

RESULTS AND DISCUSSION

The mean values for the measured traits can be found in Table1. Mean weaning weight (WW), initial weight and finish weight for the Charolais bulls and heifers respectively were 254.02 (48.28), 355.38 (51.47), 473.00 (57.88), 234.73 (46.06), 303.17 (42.02), and 389.31 (44.35) kg. The ADG_ Calc for the Charolais bulls and heifers respectively were 1.6 (0.28) and 1.09 (0.26) kgd-¹. Similarly the ADG_WW was 1.35 (0.21) and 1.11 (0.22) kgd-¹ for bulls and heifers respectively. The similarity of the ADG values was expected. The ADG_Calc value was calculated within the same time frame as the ADG_WW value. The average spans of days that ADG_Calc and ADG_WW was measured on bulls and heifers were 73, 162, 79 and 141 respectively.

Recommended durations for the measurement of ADG proposed in the literature are 63 d(Wang et. al., 2006), 70 d(Archer et. al., 1997), 84 d(Swiger and Hazel, 1961; Lui and Makarechian 1993a,b) and 112 d(Franklin et. al., 1987; Kemp, 1990; Brown et. al., 1991). Both measures of ADG fit the criterion or are longer than proposed.

As expected the mean values for all of the RFI calculations were 0. The DMI values for both genders were the largest relative to the decreased measurements. As the animals progressed through the feeding study their BW increased so they needed more feed to maintain their condition as well as stimulate growth. Similar results were reported by Brown et. al.,(1991) where feed intake mean values increased form day 84 to day 122 and day 112 to day 140. All of the measurements will not be exhaustively listed but can be found in Table 1. The DMI for the Charolais bulls and heifers, respectively were 9.26 (1.37) and 7.81 (1.0) kgd-1.

The partial correlations coefficients amongst ADG Calc and ADG WW was r = 0.61 (P < 0.0001). Much stronger relationships were reported by Brown et. al., (1991) of 0.93 (P < 0.001) amongst ADG measured over 112 d and ADG measured over 140 d, respectively. Wang et. al., (2006) reported Spearman rank correlations of 0.87 (P <(0.01) between ADG measured over 63 d when compared to ADG measured over 90 d. The decreased values seen in this population may be due to the experience that the calf had post weaning. The other aforementioned authors measured BW on cattle that had been weaned and therefore would be expected to show less variation. The ADG WW was measured from weaning to the last day of the post weaning gain test. This measurement incorporates more variation into the measurement attributable to the experiences that the calf has during and following weaning.

Table 1. BW, DMI, RFI, and ADG Mean Values for Growing Charolais Bulls and Heifers

	Bull;	Bull; n = 519		; n = 451
Item	Mean	Std Dev	Mean	Std Dev
Weaning Weight, kg	254.02	48.28	234.73	46.06
Initial Weight, kg	355.38	51.47	303.17	42.02
Finish Weight, kg	473.00	57.88	389.31	44.35
Days on Study, d	73.43	3.11	78.54	8.89
Gain to Feed, kg/dmi	0.17	0.03	0.14	0.03
ADG_Calc, kg/d	1.60	0.28	1.09	0.26
ADG_WW, kg/d	1.35	0.21	1.11	0.22
RFI_CALC, kg/d	0.00	0.64	0.00	0.66
RFI_WW, kg/d	0.00	0.64	0.00	0.66
DMI, kg/d	9.26	1.37	7.81	1.06
0-10 DMI, kg/d	8.18	1.34	7.05	1.32
0-20 DMI, kg/d	8.62	1.35	7.30	1.22
0-30 DMI, kg/d	8.91	1.41	7.40	1.18
0-40 DMI, kg/d	9.06	1.43	7.49	1.15
0-50 DMI, kg/d	9.16	1.43	7.56	1.14
0-60 DMI, kg/d	9.22	1.41	7.67	1.13

Averages for DMI were calculated on 10 day increments and compared to DMI. The partial correlation coefficients of the reduced measurements amongst DMI are shown in Table 2. The value that had the greatest correlation with DMI was 0-60 DMI (r=0.99, P < 0.0001) and the metric that had the lowest correlation was 0-10 DMI (r = 0.85, P < 0.0001). The 0-30 DMI was strongly correlated to DMI (r = 0.95, P < 0.0001). For periods measuring feed intake of greater than 30 d, the changes in the correlations of the averages were minimal. Wang et. al., (2006) reported Spearman rank correlation of 0.93 (P < 0.01) between DMI measured over 35 d and DMI measured over 91d. Archer et. al.,(1997) reported a phenotypic correlation of 0.87 amongst feed intake measured over 35 d and feed intake measured over 119 d. This suggests that 30 to 35 days of daily feed intake measurement is the minimum needed to accurately estimate feed intake.

The DMI values estimated over the reduced time-spans were used to calculate RFI along with the ADG Calc to identify the correlations amongst these values with the standard measurement of RFI. The partial correlation coefficients of the RFI Calc amongst the RFI Calc calculated with DMI measurements over reduced time-spans are shown in Table 3. The correlation between RFI Calc and RFI Calc 30 was r = 0.89 (P < 0.0001) and when DMI was estimated over and increased period of 10 d more, the correlations improved to r = 0.93 (P < 0.0001). Wang et. al., (2006) suggested that 63 d was minimum period needed to measure RFI due to the correlation of 0.90 between 63 d and 91 d measurement periods. This proposal would shorten the post weaning test by 7d. However the duration of a post-weaning test is not limited by the measurement of feed intake, but rather by the time period needed to accurately estimate gain (Archer et. al. 1997). The analysis in the present study suggests that it is feasible to measure DMI on growing beef cattle for 35 to 40 d within the longer measurement period required to accurately estimate gain. If linearity of DMI and ADG can be inferred and constant through careful management, it may be feasible to use such an approach to calculate accurate RFI values using the shorter DMI measurement period.

The ADG_WW was used in the RFI model and compared to the RFI_Calc calculation. The correlation amongst RFI_Calc and RFI_WW was r = 0.96 (P < 0.0001), RFI was then calculated using the ADG_WW measurement and the measures of DMI over the reduced time-spans. The correlation between RFI_Calc and RFI_WW_30 was r = 0.86 (P < 0.0001), and similarly with RFI_WW_40 was r = 0.90 (P < 0.0001). The complete sets of correlations are shown in Table 4. The relationships amongst these values with the control was weaker than when RFI was calculated with ADG_Calc, most likely due to the relationships amongst ADG_WW and ADG_Calc although the differences appear to be marginal.

Reducing the duration of time that animals need to be housed in pens equipped with feed intake measuring equipment has advantages (Wang et. al., 2006). Testing facilities would be able to measure more cattle and reduce

Table 2.	Partial	correlation	coefficients	of DMI	measures
from the	Error S	SCP Matrix	x / Prob > r		

	DMI
DMI	1
0-10 DMI	0.85
	<.0001
0-20 DMI	0.91
	<.0001
0-30 DMI	0.95
	<.0001
0-40 DMI	0.97
	<.0001
0-50 DMI	0.98
	<.0001
0-60 DMI	0.99
	<.0001

Table 3. Partial correlation coefficients of RFI_Calc compared to the RFI model calculated with the Reduced Intake measurements from the Error SSCP Matrix / Prob > | r|

	RFI_CALC
RFI CALC	1
-	
RFI Calc_10	0.73
_	<.0001
RFI Calc_20	0.83
_	<.0001
RFI Calc_30	0.89
	<.0001
RFI Calc_40	0.93
_	<.0001
RFI Calc_50	0.96
_	<.0001
RFI Calc_60	0.98
	<.0001

Table 4. Partial Correlation Coefficients of RFI_Calc Compared to the RFI Model Calculated ADG_WW and the Reduced Intake Measurements from the Error SSCP Matrix / Prob > |r|

	RFI_CALC	RFI_WW
RFI_CALC	1	0.96
		<.0001
RFI_WW	0.96	1
	<.0001	
RFI WW_10	0.71	0.70
	<.0001	<.0001
RFI WW_20	0.80	0.81
	<.0001	<.0001
RFI WW_30	0.86	0.88
	<.0001	<.0001
RFI WW_40	0.90	0.93
	<.0001	<.0001
RFI WW_50	0.93	0.96
	<.0001	<.0001
RFI WW_60	0.95	0.98
	<.0001	<.0001

the data collection costs. Other cost associated with testing cattle such as feed and yardage at the facility would be unchanged. Given the constraints and need for high accuracy in estimating, DMI and ADG, ultimately the duration of the test is not likely to change since the measurement of ADG is the limiting factor and the measurement period needs to be at least 70d. There are considerations that need to be made through a need to maintain the animals' environment throughout the testing period, ensuring linearity of both DMI and ADG. Accuracy was lost when calculating RFI with ADG_WW. The effects were minimal, but present.

IMPLICATIONS

There is opportunity to change the current paradigm in which we measure DMI and ADG for the calculation of RFI. The suggested strategies allow for a minimal amount of error entered into the calculation relative to the control, specifically when measuring ADG. These strategies would not be recommended for experiments identifying the differences amongst animals of different RFI classes. These strategies would however allow for testing of multiple progeny in estimating sire RFI with similar accuracy with a lower cost of the measurement, ultimately allowing the industry to test more animals for a similar cost.

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Effects of level and source of supplemental trace minerals on growth responses of beef calves received from New Mexico ranches

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ABSTRACT: Five hundred and twenty-seven crossbred cattle (initial BW 255 ± 14.5 kg) from New Mexico ranches were used in a randomized complete block design with a $2 \times$ 2 factorial arrangement of treatments. The objective of this study was to investigate the effects of Zn, Mn, Cu, Co, and Se source (Inorganic or Organic), as well as dietary inclusion levels (1X NRC recommended levels or 3X recommended levels) on health and overall performance of stressed cattle during a 56 d receiving trial. On d 0, cattle were separated by load weighed and randomly assigned to pen. Pens were randomly assigned to one of four supplemental treatments: 1) Supplement formulated to provide 100% of the NRC recommended levels of Zn, Mn, Cu, Co, and Se from inorganic sources (INO1X); 2) 100% of NRC recommended levels of Zn, Mn, Cu, Co, and Se from a 50:50 combination of inorganic and organic sources (OR1X); 3) Three times NRC recommended levels of Zn, Mn, Cu, Co, and Se from all inorganic sources (INO3X), 4) 3X NRC levels of Zn, Mn, Cu, Co, and Se from a 50:50 combination of inorganic and organic sources (OR3X). Cattle were again weighed on d 28 and 56. Cattle were assessed daily for symptoms of Bovine Respiratory Disease and treated accordingly. No interaction (P > 0.16) of mineral source by level was detected for BW, ADG, or G:F. Body Weight did not differ (P > 0.22) by mineral source or level at d 0, 28 or 56. Average daily gain did not differ (P > 0.21) across treatment for d 0-28. Mineral level did not affect (P > 0.53) ADG from d 29-56 or d 0-56. However cattle fed inorganic mineral tended (P = 0.06) to have greater ADG than cattle fed organic mineral for d 29-56 as well as d 0-56. Gain to feed ratio did not differ (P > 0.12)by treatment or level for d 0-28, d 29-56 or d 0-56. Mineral source, level or source by level had no affect (P > 0.20) on DMI for d 0-28. No difference (P > 0.28) in morbidity was observed across treatment. Mineral source or level did not change BW, ADG, or health status.

Key words: Feedlot, Growth, Health, Level, Mineral

INTRODUCTION

Trace minerals, including copper, manganese, cobalt, selenium and zinc are essential for growth and health in both humans and animals. Shipping and marketing stress can deplete reserves of critical trace elements as cattle arrive at the feedlot, while disease and decreased intake levels can compromise the ability of the animal to restore adequate levels of essential nutrients to help ensure adequate immune function and reduce incidence and severity of disease. Secondary to stress, poor nutrition prior to or upon arrival to the feedlot will also suppress immune function and subsequently decrease performance (Galyean et al., 1999). Most often calves entering the feedlot have reduced intake, which has been shown to be related to a higher incidence of morbidity (Hutcheson and Cole, 1986). Providing effective levels of these micronutrients to highly stressed calves entering the feedlot continues to be a challenge due to the negative effects of multiple stressors on immune function and nutrient intake.

Another consideration is the poor forage conditions resulting from drought which calves may experience prior to weaning and shipping to the feedlot may deplete available reserves of essential trace minerals and thereby compromise the ability of calves to have an effective immune response to novel pathogens that may be encountered when calves are received into the feedlot. Cattle that become morbid during the receiving period have been shown to have lower ADG when compared to healthy cattle (Graves et al., 2013). The ability to rapidly replete essential trace minerals during the initial weeks following arrival into the feedlot has the potential to decrease morbidity and subsequently improve performance during the feedlot receiving period. Our hypothesis is that supplemental mineral from organic sources at levels above the current NRC recommendations will improve animal health and growth performance. The objective of this experiment was to evaluate the effects of supplemental trace mineral level and source on growth performance, intake and health in calves that have been derived either from directly from New Mexico ranches during the initial 56 days following arrival into the feedlot.

MATERIALS AND METHODS

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

Five hundred and twenty-seven steers and heifers (initial BW 255 ± 14.5 kg) originating from six New Mexico ranches was used in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Research was carried out at the Clayton Livestock Research Center (**CLRC**) in Clayton, New Mexico. Upon arrival, cattle were placed in

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receiving pens overnight with ad libitum access to water and long-stem wheat hay. The basal diet was formulated to meet or exceed the requirements for newly received cattle (NRC, 2000).

Upon arrival, cattle were placed in receiving pens overnight and had ad libitum access to water and a highly palatable starter ration, and wheat hay. Approximately, 20 h after arrival (d 0), cattle were vaccinated for infectious bovine Rhinotracheitis (**IBR**) /parainfluenza-3 virus (**PI3**)/ bovine viral diarrhea/bovine respiratory syncytial virus (Bovi-Shield Gold 5 and Inforce-3, Zoetis Animal Health, Florham Park, NJ) and treated for parasites with Doramectin (Dectomax 1%, Zoetis Animal Health). Cattle were implanted with a Synovex C implant (100 mg progesterone and 10 mg estradiol benzoate per implantation, Zoetis Animal Health).

On d 0 individual BW and rectal temperature were taken during processing and an individual ear identification tag was given to each steer. Additionally, 9 mL of blood was collected from the jugular vein into a Vacuette tube with serum clot activator (Greiner Bio-One, Kremsmuenster, Austria). Calves were dehorned and castrated as needed. Cattle were provided a metaphylactic treatment with 5.0 mL tildipirosin (Zuprevo, Merck Animal Health, Summit, NJ). Cattle were then bled and weighed again on d 28 and 56. Cattle were fed twice daily at 0700 h and 1300 h one of 2 diets over the course of the study. Upon treatment with metaphylaxis cattle were placed on a 5 day moratorium before they were eligible for second treatment. Cattle were observed throughout the daylight hours for symptoms of BRD and treated accordingly. Cattle were pulled from their pen based on observed signs of sickness (e.g. lethargy, nasal discharge or abnormal activity). Cattle pulled were moved to processing facility where BW and rectal temperature were measured. Cattle that had lost BW since last weighing or had a temperature of ≥40.5 °C or any other abnormal respiration pattern were treated. When required, cattle were administered with florfenicol and fluninixin meglumine (13.2 mL/100 kg BW, Resflor Gold, Merck Animal Health). All animals were returned to pen after treatment. If after 72 hours a second treatment was required, cattle were administered enroflaxacin (12.5 mL/100 kg BW, Baytril, Bayer Animal Health, Shawnee Mission, KS).

Cattle were separated by load and randomly assigned to pen. Pens were randomly assigned to one of four supplemental treatments: 1) Supplement formulated to provide 100% of the NRC (2000) recommended levels of Zn, Mn, Cu, Co, and Se from inorganic sources (INO1X); 2) 100% of NRC (2000) recommended levels of Zn, Mn, Cu, Co, and Se from a 50:50 combination of inorganic and organic (Availa-4, ZinPro Corp., Eden Prairie, MN and selenomethionine) sources (OR1X); 3) Three times NRC (2000) recommended levels of Zn, Mn, Cu, Co, and Se from all inorganic sources (INO3X), 4) 3X NRC levels of Zn, Mn, Cu, Co, and Se from a 50:50 combination of inorganic and organic (Availa-4, ZinPro Corp., Eden Prairie, MN and selenomethionine) sources (**OR3X**). Each treatment consisted of 12 pens per treatment. Cattle were feed twice daily at 0700 and 1300 and all pens had ad libitum access to water from automatic water tanks.



Figure 1. Day 29 – 56 DM intake. Level x source interaction (P = 0.001). Columns with different superscripts differ ^{abc}P < 0.01; ^{de} P < 0.05.

Composition of the diets fed over the 56 d experiment is provided in Table 1.

Estimates of the quantity of unconsumed feed remaining in the feed bunks were made at approximately 0700 daily, and feed delivery was managed to minimize accumulation of orts in feed bunks. Weekly samples of feedstuffs and complete diets were obtained to analyze for dry matter. Samples were sent to Servi-Tech Laboratories in Amarillo, TX for DM, N, NDF, ADF, and complete mineral analysis.

Growth performance and intake data was analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Model included the influence of source of mineral, level and source × level interactions. Block was used as the random statement and pen was the experimental unit. Health data were analyzed using the PROC GLIMMIX procedure of SAS. Data are presented as least squares means and differences were considered significant at $P \le 0.05$ and as a trend at $P \le 0.10$.

RESULTS AND DISCUSSION

Effects of trace mineral source and level on BW, ADG and G:F of calves during the 56 d receiving period are presented in Table 2. No mineral source by level interaction (P > 0.16) was observed for BW, ADG, or G:F therefore interaction effects were removed from the model and the main effects of mineral source and mineral level are reported. The source of mineral or level of inclusion in the diet did not affect (P > 0.22) BW at d 0, 28 or 56. Mineral inclusion level did not affect (P > 0.21) ADG for d 0-28, 29-56 or 0-56. This data disagrees with results from Spears and Kegley (2002) who observed an increase in ADG for cattle fed an increased level of Zn. Ward and Spears (1997) also observed similar results when cattle fed increased levels of copper had an increase in ADG. Mineral source did not affect (P = 0.45) ADG for d 0-28. However cattle fed inorganic minerals tended (P = 0.06) to have higher ADG when compared to cattle fed organic sourced minerals for d 29-56 and d 0-56.

rule i. culculated alet composition of a cultion	Table 1.	Calculated	diet com	position	by t	reatment
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		Treatr	nents	
Item	Inorganic 1X	Inorganic 3X	Organic 1X	Organic 3X
Ingredient, DM basis				
Corn gluten feed, %	44.16	44.16	44.16	44.16
Corn, grain cracked, %	22.40	22.41	22.40	22
Corn stover, %	20.95	20.95	20.95	21
Corn dried distillers grains with soluble, %	5.1	5.1	5.1	5.0
Trace mineral supplement				
Corn dried distillers grains with soluble, %	4.87	4.88	4.87	5.00
Limestone, %	1.78	1.78	1.78	1.77
Urea nitrogen,%	0.34	0.34	0.34	0.34
Sodium Chloride, %	0.27	0.27	0.27	0.27
Vitamin E-50, %	0.041	0.041	0.041	0.041
Bovatec, %	0.03	0.03	0.03	0.03
Selenium, %	0.016	0.016	0.016	0.016
Zinc sulfate, %	0.008	0.024	0.004	0.012
Manganese Sulfate,%	0.006	0.017	0.003	0.008
Copper Sulfate,%	0.004	0.001	0.002	0.006
Vitamin AD Rovimix,%	0.0004	0.0004	0.0004	0.0004
EDDI,%	0.00007	0.00021	0.00007	0.0002
Cobalt Sulfate, %	0.00003	0.00009	0.00002	0.00005
Availa 4, %			0.028	0.085
Nutrient composition				
NE _m , Mcal/kg	1.86	1.86	1.86	1.86
NEg, Mcal/kg	1.26	1.25	1.26	1.25
CP,%	17.83	17.81	17.82	17.80
Ca, %	0.77	0.77	0.77	0.77
P, %	0.69	0.68	0.68	0.68
Co, ppm	0.10	0.57	0.29	0.87
Cu, ppm	9.93	29.79	10.14	30.43
Mn, ppm	19.93	59.77	18.01	54.03
Zn, ppm	29.87	89.58	29.74	89.19
Se, ppm	0.10	0.29	0.10	0.29

Table 2.	Effects of minera	I source and	l level on BW	, ADG	and G:F
				2.41	

	Mineral	Source		Minera	l Level	_		P-Va	lue
Item	Inorganic	Organic	SEM ¹	1X	3X	SEM ¹	Source	Level	Source x Level
BW, kg									
D 0-28	255	253	11.2	257	251	11.2	0.78	0.42	0.16
D 29-56	284	280	13.3	283	281	13.3	0.57	0.75	0.18
D 0-56	320	309	12.9	317	312	12.9	0.22	0.61	0.21
ADG, kg									
D 0-28	1.04	0.95	0.15	0.92	1.06	0.15	0.45	0.21	0.85
D 29-56	1.27	1.05	0.13	1.20	1.13	0.13	0.06	0.53	0.91
D 0-56	1.15	0.99	0.07	1.06	1.10	0.07	0.06	0.65	0.91
G:F, kg									
D 0-28	0.16	0.15	0.02	0.15	0.17	0.02	0.52	0.26	0.80
D 29-56	0.16	0.14	0.01	0.16	0.14	0.01	0.13	0.42	0.54
D 0-56	0.16	0.14	0.01	0.15	0.15	0.01	0.12	0.75	0.42



Figure 2. Day 0 - 56 DM intake. Level x source interaction (P < 0.001). Columns with different superscripts differ ^{abc}P < 0.01; ^{de} P < 0.05.

These results also disagree with Spears and Kegley (2002) who observed no difference in ADG for cattle fed organic sources of Zn versus cattle fed inorganic sources of Zn. Mineral source or level did not affect (P > 0.12) G:F across the 56 d trial. Our results disagree with Aloha et al. (2005) that reported feed efficiency improved when cattle were fed organic sources of copper, zinc and manganese as compared to inorganic sources.

Mineral source, dietary inclusion level or source by level interaction had no effect (P > 0.18) DMI for d 0-28. Figure 1 illustrates the differences in DMI across treatments for d 29-56. Cattle on the OR1X and INO3X treatments had higher (P < 0.05) DMI than cattle fed OR3X and INO1X. Figure 2 illustrates the differences in DMI across treatments for d 0-56. As seen in the d 29-56, similar results exist for d 0-56, in that cattle on the OR1X and INO3X treatments had higher (P < 0.05) DMI than cattle fed OR3X and INO1X. Figure 3 depicts the differences in overall morbidity across treatments for the 56 d study.

No differences (P > 0.281) in morbidity across treatment existed. With the overall morbidity being low (6.1%) the potential exist that differences could be detected with there had been a higher incidence of morbidity.

IMPLICATIONS

From this study, it can be concluded that mineral source and mineral level have no effect on BW, ADG or G:F. These results agree with previous data that have shown equivocal results for cattle fed differing sources of minerals. From these results we can conclude that the NRC recommend level for inclusion of trace minerals provides adequate levels to maintain proper immune function and performance.



Figure 3. Percent morbidity across treatments (P > 0.28)

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A preliminary report on the impact of cryopreservation diluent on ram sperm physiology and cervical artificial insemination¹

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INTRODUCTION

ABSTRACT: A meta-analysis of our fertility trials using Chi-square demonstrated that use of the skim-milk-egg yolk (SMEY) diluent resulted in significantly greater fertility with the SMEY diluent (42%) compared with the TRIS diluent (15%; P < 0.05). Therefore, we hypothesized that cryopreservation diluent (SMEY or TRIS) utilized in these trials may have a strong impact on ram sperm physiology. To test this, ram semen samples (n = 3) were collected and aliquots diluted to 600 x 10⁶ sperm/mL in either SMEY or TRIS diluents. The samples were cooled to 5 °C over 2 h. Aliquots (200 x 10⁶ sperm) were removed and diluted in 39 °C capacitation media and maintained at this temperature for the duration of the capacitation analyses. Aliquots of the capacitating samples were removed after 180 min of incubation for computer automated semen analysis (CASA) and for flow cytometry (analysis of membrane integrities, intracellular calcium, mitochondrial activity, and phospholipid organization). The analyses were repeated after incubation of the samples in the cryopreservation diluents for 24 h at 5 °C. Samples diluted in SMEY had a greater proportions of sperm with functioning mitochondria (58% vs 41%) and were better able to accumulate intracellular calcium compared with TRIS samples (12% vs 5%), respectively (P ≤ 0.01). Diluent did not affect plasma membrane integrity or apoptotic characteristics (P > 0.05). Diluent and holding time did not affect the proportion acrosome reacted sperm or the membrane phospholipid organization but SMEY had greater proportions of motile and progressively motile sperm (31 and 20%, respectively) compared with TRIS (21 and 10%, respectively; P < 0.001). These results demonstrate that diluent influences the physiology of ram sperm and in particular that TRIS may modulate accumulation of intracellular calcium and mitochondrial activity; both of which are necessary for capacitation to occur. However, additional analyses are required in order to fully understand the impact of diluent on sperm physiology and fertility.

Key words: artificial insemination, milk, semen extender, spermatozoa, TRIS

Numerous factors (e.g. ram, ewe, estrous synchronization protocol, timing of AI, semen quality, semen diluent) affect fertility rates when using frozen-thawed ram semen for cervical AI. To address one aspect of this issue Paulenz et al. (2002) compared diluents and demonstrated that storing ram sperm in TRIS at 5 °C preserved viability (plasma membrane and acrosomal membrane integrity) better compared to SMEY diluent. However, when fertility trials were performed with the liquid cooled semen the samples stored in SMEY had greater ewe fertility (64 vs 54% lambing) compared to the samples stored in the TRIS diluent (Paulenz et al., 2003). Similarly, we too were able to achieve greater fertility (unpublished data) with cervical insemination when SMEY was used (42%) compared with the fertility achieved when TRIS was used (15%; P < 0.05). Paulenz et al. (2002, 2003) concluded their analyses to determine in vitro sperm quality were not appropriate for assessing fertility in their field trial. In order to address this knowledge gap, and understand why we too are able to improve ewe fertility rate when using the milk diluent, we investigated the effects of these diluents on the motility and physiology of ram sperm that were incubated in media that induces capacitation. We believe that by observing sperm under these conditions and over time a more appropriate estimation of sperm quality and fertilizing potential can be estimated.

MATERIALS AND METHODS

Semen Collection, Preservation, and Capacitation Induction

All procedures were approved by the University of Wyoming and the USDA-NAGP Institutional Animal Care and Use Committee. Semen was collected from 3 sexually mature rams (Rambouillet) and the sperm concentration and total motility were determined. All samples had \geq 80% total motility. Each sample was then then split and diluted to 600 x 10⁶ sperm/mL in a SMEY diluent (Paulenz et al, 2003) or

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a TRIS diluent (Sanchez-Partida et al., 1998). Samples were then placed in a beaker with 37 °C water and cooled to 5 °C within 2 h of collection. The samples were held at this temperature for the duration of the experiments.

Aliquots (200 x 10^6 sperm) of the cooled samples were removed, diluted in capacitation media (Grasa et al., 2009) and incubated at 39 °C in a humidified environment. After 180 min of incubation aliquots were removed and analyzed to determine the sperm motility and physiologic characteristics using computer automated semen analysis (CASA) and flow cytometry, respectively. The capacitation incubation and related analyses were repeated 24 h later using additional aliquots of the samples that were stored at 5 °C.

Motility Analyses

Motility analyses were performed with CASA (HTM-IVOS Version 14, Hamilton Thorne Bioscience, Beverly, MA, USA) using the system parameters described by Purdy et al. (2010). A minimum of 500 sperm from at least 5 fields were analyzed for each sample.

Flow Cytometry

Aliquots of sperm (10 x 10⁶) were diluted in phosphate buffered saline (ph 7.2, 300 mOsm) and stained to detect acrosomal integrity using FITC-PNA (Flesch et al., 1998), intracellular calcium using Fluo-3 AM (Purdy and Graham, 2004), plasma membrane phospholipid organization using Merocyanine 540 (M540; Guthrie and Welch, 2005), and mitochondrial activity using Mitotracker Green (Garner et al, 1998) in separate analyses. The samples were counterstained with either propidium iodide or Yo-Pro 1, depending on the fluorescence spectra, to identify sperm with damaged membranes. The flow cytometry was performed using a Cyan-ADP flow cytometer (Beckman Coulter, Miami, FL, USA) with a 488nm argon laser at 150mW of power. The filters included a 95% reduction filter, a 545nm dichroic long pass filter, a 640 nm dichroic long pass filter a 530/40 nm band pass filter (FITC-PNA, Fluo-3 AM, Mitotracker Green and Yo-Pro 1 fluorescence) and a 613/20 nm band pass filter (M540, Propidium iodide fluorescence). At least 10,000 sperm were analyzed per sample.

Statistical Analyses

Chi-square analysis was used to determine differences in pregnancy rate for sperm frozen in SMEY and TRIS diluents (SAS, 1985). Percentage data was transformed using arcsine (SAS, 1985). ANOVA (PROC GLM) was used to determine differences in the motility and sperm physiology values. The model included the effects of cryopreservation diluent (SMEY or TRIS), holding time (0 or 24 h), ram, and appropriate interactions (SAS, 1985). The means were separated using the Duncan test (SAS, 1985).

RESULTS AND DISCUSSION

Paulenz et al. (2002) observed that dilution of ram sperm in TRIS diluents resulted in greater proportions of sperm with intact acrosomes, intact plasma membranes and cells that had not capacitated following incubation compared with samples that were held in milk diluents. In contrast, we observed that the type of diluent (SMEY or TRIS) did not affect the proportion of acrosome reacted (PNA), plasma membrane intact (PMI) or the membrane phospholipid organization (M540; Table 1) which can be correlated with capacitation (Guthrie and Welch, 2005). Likewise, the proportion of apoptotic-like sperm (Yo-Pro+) and the motility characteristics, excluding total and progressive motility, were not affected by either diluent.

However, we did observe that, similar to Paulenz et al (2002), samples diluted with the SMEY diluent appeared more able to demonstrate capacitation–like characteristics based on the greater proportion of sperm with high intracellular calcium and active mitochondria (Visconti et al., 1998). Motility analyses further identified differences in sperm quality attributed to the cryopreservation diluent. Use of TRIS diluent suppressed total and progressive motility compared with the samples diluted in the SMEY diluent (Table 1). More importantly, observation of the effects of the diluents on the quality of motility revealed that the TRIS diluent may be inducing hyperactivation-like characteristics (increased VCL and ALH, and decreased STR and LIN;

Table 1. Characteristics of ram sperm following dilution in either milk or TRIS based medium, cooling to 5 °C and incubation at 39 °C for 3 h to induce capacitation. Analyses were performed after initial cooling and again 24 hours later. Values are the mean of the incubation times by diluent.

Characteristic	MILK	TRIS	SEM	Р
PNA (%)	2.8	3.6	1.0	0.9
PMI (%)	34	32	2.0	0.6
M540 (%)	2.4	2.9	0.4	0.3
Yo-Prol (%)	18	25	3.0	0.2
Fluo (%)	12	5	1.2	0.003
Mito (%)	58	41	2.2	.01
Motile (%)	31	21	1.6	<.001
PROG (%)	20	10	1.6	<.001
VCL (µm/s)	182	205	7.1	.09
VAP (µm/s)	118	106	3.5	.02
VSL (µm/s)	100	81	3.9	.01
ALH (µm)	6.8	8.5	0.4	.17
STR (%)	83	77	1.3	.06
LIN (%)	55	43	2.2	.06

¹PNA: Acrosome reacted sperm; PMI: Plasma membrane intact; M540: Merocyanine positive/ phospholipid disarray; Yo-Pro1: Non-apoptotic; Fluo: sperm positive for intracellular calcium; Mito: sperm with functioning mitochondria; Motile: total motility; PROG: sperm with progressive motility; VCL: curvilinear velocity; VAP: Average cell path velocity; VSL: Straight line velocity; ALH: lateral head amplitude; STR: straightness of motion; LIN: linearity of motion. Table 1) as described by Kaula et al. (2009) and McPartlin et al. (2009). Under in vivo conditions hyperactivation requires increased concentrations of intracellular calcium (Visconti et al., 1998) which was not observed in the TRIS samples. TRIS is known to inhibit accumulation of intracellular calcium (Alonso et al., 1979; Turlapaty et al., 1979) and inhibit mitochondrial activity (Good et al., 1966) which explains why the samples diluted with the SMEY diluent were able to have elevated levels of intracellular calcium and active mitochondria by comparison to the TRIS samples. We believe this may indicate that the SMEY diluent allows capacitation to occur in a manner similar to in vivo conditions whereas the TRIS diluent may bypass capacitation all together and induce the sperm to hyperactivate instead (McPartlin et al., 2009). Like McPartlin et al. (2009) we believe that these findings demonstrate that the capacitation and hyperactivation processes may be independent rather than sequential when analyzed under in vitro conditions.

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Temporal patterns of metabolites, metabolic hormones, and progesterone concentrations and lambing rates in Rambouillet ewes selected for high and low reproductive rate¹

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INTRODUCTION

ABSTRACT: The objectives of this study were to evaluate temporal patterns of metabolites, metabolic hormones, and progesterone concentrations during gestation, and lambing traits in Rambouillet ewes from lines selected for high (HL) and low (LL) reproductive rates. Lines of ewes had been selected for reproductive rate for over 44 yr. Twenty-seven HL and 22 LL ewes were single-sire mated over an 18-d breeding season in November. Jugular venous blood samples were collected from each ewe on d 30, 60, 90, and 120 of gestation. Pregnancy rates and litter size (number lambs per ewe) were obtained at lambing. Body weight of each lamb was recorded at birth and before suckling. Serum samples from ewes were assayed for glucose (GLUC), NEFA, cortisol, triiodothyronine (T3), thyroxine (T4), IGF-1, and progesterone (P4) concentrations. Temporal concentration patterns of metabolites, metabolic hormones, and P4 for 20 HL and 15 LL ewes were normalized to lambing date within \pm 1-wk period of the sampling day. Pregnancy rate at lambing did not differ (P > 0.05) between HL (92.6%) and LL (77.3%) ewes. Litter size was greater ($P \le 0.05$) in HL ewes than in LL ewes. Even though HL ewes had greater ($P \le 0.05$) total birth weight per ewe than LL ewes, lambs from LL ewes were heavier ($P \le 0.05$) than those of HL ewes. Temporal concentration patterns of GLUC, NEFA, cortisol, T3, T4, T3:T4 ratios, and IGF-1 did not differ between HL and LL ewes. There was an interaction ($P \le 0.05$) of line and d of gestation for P4 concentrations. P4 in HL ewes increased to a greater concentration from d 60 to d 120 than in LL ewes over this period. In conclusion, the physiological mechanism(s) for this divergence in reproductive rate in the Rambouillet lines does not appear to be directly related to changes in energy-related metabolites or metabolic hormones. However, selection appears to have altered systemic P4 concentrations after d 90 of pregnancy between the two lines of ewes. Whether this is caused by a change in luteal and(or) placental P4 production, or by a change in catabolism of P4 between ewes of these lines requires further investigation.

Key words: metabolites, metabolic hormones, progesterone, reproductive rate, genetic selection

In 1968, Dr. P. J. Burfening of the Montana Agricultural Experiment Station began a selective breeding program to generate two lines of Rambouillet sheep with high (HL) or low (LL) reproductive rates (Schoenian and Burfening, 1990). The selection index (SI) was SI = lifetime number of lambs/ (age of ewes – 1), with HL and LL sheep bred for high or low SI values, respectively. The selective breeding of these lines was found to have resulted in diverging SI values after 23 yr of selection (Burfening et al., 1993). Determined phenotypic outcomes of using this SI in these lines of ewes include that ovulation rate, litter size, and lambs born per ewe exposed have increased in HL ewes compared to LL ewes (Schoenian and Burfening, 1990). The physiological mechanisms that are involved in causing these changes are not clear.

Nutrition and metabolism are known to affect reproduction in livestock. It is now established that nutrition affects reproduction not only by providing energy to develop and sustain the fetus or embryo directly, but also through regulation of hormones that control reproduction and impact development of the neonate (for reviews see, Robinson et al., 2006; Boland et al., 2001). Furthermore, nutritional status of ewes has been shown to interact with systemic progesterone (P4) concentrations and influence pregnancy rates (Parr et al., 1987). One could hypothesize that selection for high or low reproductive rate in ewes of these Rambouillet lines has altered the metabolism and utilization of energy-related metabolites, metabolic hormones, and(or) P4 synthesis or catabolism during gestation. The objectives of this study were to evaluate temporal patterns of metabolites, metabolic hormones, and P4 concentrations during gestation, and lambing traits in Rambouillet ewes from lines selected for high and low reproductive rates.

MATERIALS AND METHODS

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

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Animals, Nutritional Management, Breeding

Thirty HL and 27 LL nulli-, primi-, or multiparous ewes, approximately equally divided among ages 1 to 4 yr, were used in this study. Ewes were transported from the Red Bluff Research Ranch near Norris, MT to the BARTF in fall after weaning. Ewes of both lines were co-mingled and housed in open-shed lots equipped with GrowSafe bunks (GrowSafe Systems, Ltd., Airdrie, AB, Canada) throughout the study. Ewes in both lines had access to the same diets fed in the GrowSafe bunks throughout the study. Ewes were weighed on 2 consecutive d at start of study. Ewes were fed chopped-grass hay for 41 d, beginning Oct. 5, then choppedalfalfa hay for 106 d, and finally, an alfalfa-, barley-, and molasses-pelleted diet, until lambing (55 d). The as fed DM, CP, NDF, and TDN composition of the grass hay was 91.1, 7.4, 61.2, and 54%, the alfalfa hay was 89.6, 19.4, 34.4, and 54%, and the alfalfa based pellet was 90.8, 18.1, 36.8, and 55%, respectively. Ewes had free access to water and a tracemineral supplement.

The breeding season began on Nov. 9, and the diet was switched from chopped-grass to chopped-alfalfa hay 1 wk after introduction of rams. Ewes were single-sire mated for 18 d within lines, in groups of 12 to 15, to Rambouillet rams. Rams were brisket-painted before introduction to ewes and every 3 d for detection of breeding behavior. Thereafter, ewes were exposed to 1 of 2 Suffolk rams for an additional 18 d. At lambing, lambs were weighed on a hand-held scale immediately at birth and before suckling.

Blood Sampling and Assays

Jugular venous samples were collected from each ewe on d 30, 60, 90, and 120 of gestation. Samples were stored overnight at 4°C and serum was separated by centrifugation at 1,850 x g, 30 min, at 4°C. Samples were stored at -20°C until assayed.

Glucose concentration was determined in duplicate using a glucose assay kit (Infinity Glucose Hexokinase liquid reagent, Thermo Fisher Scientific, Waltham, MA). Intra- and inter-assay CV were < 10% for ewe sera pools that contained 55.5 and 83.7 mg/dL, respectively.

Concentrations of NEFA were quantified with enzymaticcolorimetric assay kits (HR Series NEFA – HR [2]; Wako Diagnostics, Richmond, VA). Intra- and inter-assay CV were 4.1 and 9.6% for serum that contained 0.291 mEq/L; and, 5.3 and 11.7%, respectively, for serum that contained 0.113 mEq/L.

Concentrations of cortisol were determined in duplicate by solid-phase RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA). Intra- and inter-assay CV were 3.3 and 10.1% for ewe serum that contained 26.5 ng/mL; and, 3.6 and 11.5%, respectively, for ewe serum that contained 25.5 ng/mL.

Triiodothyronine and T4 were assayed in duplicate using solid phase RIA kits (Siemens Healthcare Diagnostics Los Angeles, CA, USA) validated for sheep serum (Richards et al., 1999; Wells et al., 2003, respectively). Intra- and interassay CV for T3 were 4.4 and 14.0 %, respectively, for ewe serum that contained 1.39 ng/mL. Intra- and inter-assay CV for T4 were 5.8 and 6.4 %, respectively, for ewe serum that contained 70.0 ng/mL.

Concentrations of IGF-1 were assayed by Dr. Dennis Hallford (New Mexico State University, Las Cruces, NM) in duplicate using double antibody RIA, validated for sheep serum (Spoon and Hallford, 1989). Intra- and inter-assay CV were 8.1 and 16.3%, respectively.

Progesterone concentrations were determined in duplicate by solid-phase RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA). Intra- and inter assay CV were 8.2 and 14.8% for ewe serum that contained 2.4 ng/mL; and 2.0 and 7.4%, respectively, for ewe serum that contained 11.5 ng/mL.

Statistical Analysis

Data for pregnancy rates at lambing included 27 HL and 22 LL ewes; whereas, data for litter size of ewes that lambed included 25 HL and 17 LL ewes. There were 20 HL and 15 LL ewes that were used to normalize temporal patterns of GLUC, NEFA, cortisol, T3, T4, IGF-1, and P4 concentrations to within a 2-wk period of d 30, 60, 90, and 120 of gestation. Data for BW at the start of the study, birth weight of lambs, metabolites, metabolic hormones, and P4 were analyzed using SAS procedures (SAS Inst. Inc., Cary, NC) and significance was set at $P \le 0.05$.

Pregnancy rate at lambing was analyzed with contingency chi-square analysis. Litter size, lamb birth weight, and total lamb birth weight per ewe were analyzed by separate ANOVA using PROC GLM. The model included line of ewe. Temporal patterns of metabolites, metabolic hormones, and P4 concentrations were analyzed by separate ANOVA using the PROC MIXED model for repeated measures. The model included line, day, and the line by d of gestation interaction. Ewe within line was the subject and d of gestation was the repeated measure. Means were separated using Bonferroni's multiple comparison adjustment.

RESULTS AND DISCUSSION

At the beginning of the study, BW of HL and LL ewes, used for analyses of temporal patterns of metabolites, metabolic hormones, and P4 concentrations did not differ (P > 0.05) and averaged 51.3 ± 7.3 kg. Pregnancy rates at lambing did not differ (P > 0.05) between HL and LL ewes (Table 1). This result is similar to that reported by Schoenian and Burfening (1990) for number of ewes lambing per ewe exposed. Data for pregnancy rate at lambing in the present study may indicate that an additional 23 yr of selection has not influenced this reproductive characteristic in ewes of these lines. However, this conclusion must be qualified in that the numbers of ewes per line were low.

Consistent with the results of Schoenian and Burfening (1990), litter size in the present study was greater ($P \le 0.05$) for HL ewes than that of LL ewes (Table 1). These results indicate that an additional 23 yr of selection for high or low reproductive rate using the SI [lifetime number of lambs/(age of ewes -1)] has not increased the phenotypic divergence between ewes of these lines.

Table 1. Pregnancy rates, and least squares means for litter size, lamb birth weight, and total lamb birth weight per ewe lambing of Rambouillet ewes selected for high (HL) and low (LL) reproductive rate

	Li	ne	_			
Item	HL	LL	-	P-		
	(n)	(n)		value		
Pregnancy rate,1 %	92.6	77.3	2.32^{2}	0.13		
0,000	(27)	(22)				
Litter size ³	1.52	1.18	0.47^{4}	0.02		
	(25)	(17)				
Lamb birth weight,						
kg	4.21	4.62	0.68^{4}	0.05		
	(32)	(17)				
Total weight of						
lambs per ewe, kg	6.74	5.22	1.834	0.02		
	(20)	(15)				
Description of the second seco						

'Percentages of HL and LL ewes lambing

 $^{2}\chi = 2.32; d.f. = 1.$

3Lambs per ewe lambing.

⁴Pooled SEM.

Ewes from the HL had greater ($P \le 0.05$) total birth weight of lambs per ewe, although LL ewes had larger ($P \le 0.05$) lambs (Table 1). Greater total lamb birth weight per ewe for HL ewes could be due to the greater proportion of twins in HL ewes than in LL ewes. This conclusion is consistent with results in sheep reported by Gardner et al. (2007), in which twinning ewes had lower individual birth weights, but increased total weight of all lambs per ewe lambing.

There was no line (Table 2) or line by d of gestation interaction (P > 0.05) for concentrations of GLUC, NEFA, cortisol, T3, T4, T3:T4 ratio, and IGF-1. These results indicate that selection for high and low reproductive rate in Rambouillet sheep, using the SI [lifetime number of lambs/ (age of ewes – 1)], has not influenced systemic temporal concentrations of the metabolites and metabolic hormones assessed in the present study. Concentrations of the metabolites and metabolic hormones assessed in the present study are similar to those reported for ewes during gestation (Ward et al., 2008; Verbeek et al., 2012; Vonnahme et al., 2013).

There was a line by d of gestation interaction ($P \le 0.05$) for P4 concentrations (Fig. 1). Concentrations of P4 did not differ (P > 0.05) between HL and LL ewes on d 30 and 60. Concentrations of P4 increased ($P \le 0.05$) in both HL and LL ewes from d 60 to 90. This increase in P4 concentrations in ewes of both lines at d 90 is in agreement with Sarda et al. (1973) who reported that P4 concentrations in pregnant ewes had a marked increase in P4 from d 80 to 90 of gestation. However, P4 in HL ewes increased ($P \le 0.05$) to a greater concentration from d 60 to d 120 than P4 concentration in LL ewes.

This is the first evaluation of temporal patterns of P4 concentrations during gestation in these lines of Rambouillet ewes. The physiological mechanism(s) for this interaction in

Table 2. Least squares means of metabolic hormones and metabolites during gestation for Rambouillet ewes selected for high (HL; n = 20) and low (LL; n = 15) reproductive rate

	Lin	ne		
Item	HL	LL	SEM ¹	P-value
Glucose, mg/dL	68.9	70.2	2.5	0.45
NEFA, mEq/L	0.317	0.317	0.089	1.00
Cortisol, ng/mL	19.8	18.5	4.2	0.30
T3, ng/mL	0.780	0.813	0.092	0.77
T4, ng/mL	51.5	52.9	6.5	0.60
T3:T4	0.015	0.015	0.001	0.77
IGF-I, ng/mL	428	431	109	0.95

Pooled SEM for each row item.



Figure 1. Least squares means of progesterone (P4) concentrations during gestation in Rambouillet ewes selected for high (HL; n = 20) and low (LL; n = 15) reproductive rate. Error bars represent \pm SE of each mean. Line by d of gestation interaction; P = 0.04. Means with different superscripts differ ($P \le 0.05$).

P4 concentrations from mid- to late-gestation may be related to: 1) number of corpora lutea (CL); 2) placental mass and synthesis of P4; and(or), 3) catabolism of P4. Schoenian and Burfening (1990) reported that HL ewes had more CL per ewe than LL ewes. However, it is not likely that this interaction in P4 concentrations is due to the mass of luteal tissue because concentrations did not differ between lines until d 90. Placental mass is known to be greater in ewes carrying twins than in ewes carrying singles (Vonnahme et al., 2008). Perhaps, an increase in placental mass could result in higher P4 concentrations in HL ewes late in gestation, since there was a greater proportion of HL ewes carrying twins than LL ewes. However, Sarda et al. (1973) reported that systemic P4 concentrations did not differ between ewes carrying singles and twins. Thus, it is possible that selection for high and low reproductive rate has either increased the mechanism for P4 synthesis in HL ewes or decreased this mechanism in LL ewes, resulting in divergent P4 concentrations late in gestation between ewes in these lines. However, Berardinelli et al. (1995) reported that P4 concentrations were higher in HL than LL ewes between d 2 and d 10 of the estrous cycle. This difference was not related to number of CL in the ovary because venous ovarian concentrations of P4 did not differ between HL ewes that had 2 CL and LL ewes that had 1 CL in their ovaries (Berardinelli et al., 1995). These authors hypothesized that the clearance rate of P4 from the systemic circulation during mid-luteal phase of the estrous cycle in HL ewes was slower than the clearance rate in LL ewes. One could hypothesize that the difference in P4 concentrations that we observed late in pregnancy, between HL and LL ewes, could be a result of a change in catabolic processing of P4.

In conclusion, selection of Rambouillet ewes for high or low reproductive rate has not only caused a difference in litter size and total kg of lamb born per ewe, but it has also resulted in divergence of late-gestation P4 concentrations between lines. Results for litter size and total kg of lamb born per ewe confirm that the use of this SI has maintained a difference in reproductive rate between these lines. These phenotypic characteristics do not appear to be driven by differences in metabolites or metabolic hormones assessed in this study. Differences in temporal P4 concentrations perhaps reflect genetic differences caused by the SI that may relate to specific physiological processes affecting reproductive rate in sheep.

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Effects of GnRH and prostaglandin combined with a short progestin regimen on the synchrony of estrus and ovulation in Suffolk ewes during the breeding season

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ABSTRACT: This study was conducted to quantify the effects of GnRH and prostaglandin in conjunction with a 7-d CIDR on synchrony of estrus in comparison with a traditional synchronization protocol. Suffolk Ewes (n = 40)were randomly allotted to 1 of 5 treatments: CIDR (7 d) with administration of GnRH (Cystorelin[®], 50µg, im) at CIDR insertion and PGF2 α (Lutalyse[®], 20 mg, im) on d 6.5 (GnRH1); the GnRH1 protocol with a second injection of GnRH 30 h after CIDR removal (GnRH2); and CIDR (11 d) with administration of PGF2a at CIDR insertion and PMSG (400 iu) at CIDR removal (PMSG). Treatments 4 and 5 were utilized as controls, CIDR (7d) and CIDR (11 d) respectively. A blood sample was obtained beginning 18 h after CIDR pull, every 2 h for 38 h for serum LH analysis. Two ewes that brought on complications during the bleeding process were excluded from analysis, one in the GnRH1 group and the CIDR7 group. Mean serum concentrations of LH differed among groups (P < 0.05) from 22 to 42 h following CIDR removal. Females in the GnRH2 group exhibited the tightest window of synchrony among groups as well as the highest concentration of serum LH levels. The 2 protocols using the shorter 7 d progestin regimen and GnRH to control follicular dynamics resulted in higher estrus and ovulation rates and an acceptable window of synchrony. These results indicate that synchrony of estrus can be increased in response to a CIDR protocol when combined with administration of GnRH rather than PMSG.

Key words: CIDR, GnRH, synchronization, timed artificial insemination protocol

INTRODUCTION

Artificial insemination (AI) has been a vital tool for the genetic improvement of livestock for many years. The cattle industry, especially dairy operations have utilized AI to a greater extent than any other livestock species. There are many factors that contribute to their wide use of AI, but one of the greatest contributors is the application of effective synchronization protocols. The sheep industry has much to gain from the increased use of AI, but there are several obstacles impeding the use of AI within the industry.

Several problems that make AI less profitable and efficient are the lack of effective, consistent synchronization protocols and the difficulty of estrus detection. These problems could be minimized by the development of a timed artificial insemination (TAI) protocols that yield consistently higher conception rates and eliminate the need for estrus detection. The generally accepted method of estrus synchronization for TAI in sheep uses a 11-19 d regiment of progestin administered via a controlled internal drug release (CIDR) device, an injection of prostaglandin F2a (PGF2a) at CIDR insertion, and in some cases an injection of equine chorionic gonadotropin (eCG) in the form of pregnant mare serum gonadotropin (PMSG) at CIDR removal. The industry's current standard protocol uses PMSG or PG600® which acts like FSH and LH in the ewe's endocrine system. This property causes it to narrow the window of ovulation time when PMSG is used in synchronization protocols (Evans, 1988; Menchaca and Rubianes, 2004). A recently discovered drawback to the use of PMSG is the immune response of ewes after PMSG is administered multiple times. Maurel et al. (2003) showed that PMSG causes an immunogenic response in ewes.

The immune response to PMSG causes decreased conception rates as it is used to synchronize ewes from year to vear. The effectiveness protocols utilizing PMSG can also be very inconsistent. This is probably due in part to the immune response some ewes have to its effects. PMSG acts similarly to LH and FSH, but does not have a well-known and defined effect on the endocrine system. The functions of the PMSG alternative, GnRH, in ruminant animals are better understood. The lack of consistent results from exogenous PMSG in AI protocols may be due to additional affects it has on the reproductive system. There may also be other factors contributing to its unpredictability which could be discovered through further research. These negative aspects of PMSG point out the need to find an effective protocol that uses GnRH in the place of PMSG.

Recent research findings show a synchronization protocol which uses a 7 d CIDR, gonadotropin releasing hormone (GnRH), and PGF2a may be an effective way to synchronize ewes for TAI. These protocols u s e GnRH to dictate follicular wave development on the ovaries and to cause ovulation of dominant follicles after CIDR removal. Dickison et al. (2010) reported ewes synchronized with this protocol reached estrus from 34-40 h after CIDR removal compared to ewes synchronized with the current industry standard protocol which reached estrus from 25-68 h. This tighter window of ovulatory synchrony is very desirable for ewes to be inseminated at a fixed time. Therefore the objective of this study is to determine the time of ovulation of three experimental groups and two control groups of ewes synchronized using different protocols. Time of ovulation will be determined by the level of luteinizing hormone (LH) found in blood samples collected from the experimental ewes.

MATERIALSAND METHODS

The ewes utilized in this experiment were managed according to the guidelines of the Angelo State University Institutional Animal Care and Use Committee. Forty multiparous Suffolk ewes were randomly assigned to one of five treatment groups.

Each group was synchronized using a different protocol with each protocol utilizing a CIDR. Protocols were started at respective times so that CIDR removal was simultaneous for each group. The first group (GnRH1) was synchronized using the following protocol: on d 1 a progestin implant (CIDR containing 0.3 g progesterone) was inserted intravaginally and a GnRH injection (Cystorelin[®] 50 ug/mL) was administered IM, on d 6.5 an IM injection of prostaglandin (Lutalyse® 5 mg/mL) was given and on d 7 the CIDR was removed. The second group (GnRH2) was synchronized using the same protocol as Group 1 with an additional GnRH injection 38 h following CIDR removal. The third group (PMSG) was synchronized using the industry's current standard protocol which is: on d 1 a progestin implant (CIDR containing 0.3 g progesterone) was inserted intravaginally and an intramuscular (IM) injection of prostaglandin (Lutalyse 5 mg/mL) was given and on d 11 the CIDR was removed and an injection of PG600 was administered. The fourth and fifth groups (CIDR7 and CIDR11, respectively) served as control groups for the experimental protocols using 7 and 11 d CIDRs.

Blood collections via jugular venipuncture began 18 h following CIDR removal and were repeated every 2 h for 38 h consecutively. Approximately 5 mL of blood was collected from each ewe and placed directly on ice. The blood samples were then centrifuged and frozen at -80°C until the time of the LH assay. Ewes were observed for signs of estrus from the time of CIDR removal to the end of the bleeding period. Mark times were recorded for each ewe as soon as they were in standing estrus and the ewes had a definite mark from the rams equipped with breeding harnesses.

Concentrations of LH in the samples were determined by a double antibody radioimmunoassay (RIA) as described by Recabarren et al. (1996) analyzed for LH concentration to estimate time of ovulation. On d 1, 500 uL of 1% phosphate buffered saline (PBS) and egg white (PBS-EW) were added to the non-specific binding (NBS) and the 0 standard tubes. Twohundred microliters of standard and 300 uL of 1% PBS-EW were added to each standard tube. Three-hundred microliters of 1% PBS-EW and 200 uL of each sample were put into each unknown tube. The reference preparation tubes contained 300 uL of 1% PBS-EW and 200 uL of reference preparation.

The primary antibody anti-oLH was diluted with PBS-EDTA and normal rabbit serum (NRS) at a 1:400 ratio. Two hundred microliters of the antibody was then added to all tubes except the NSB and total count tubes. A trace consisting of 100 uL of ¹²⁵I-oLH (20,000 CPM/100 uL diluted in 0.1% PBS-EW) was added to all tubes. Then the tubes were vortexed and incubated for 24 h at 4°C for 48 to 72 h. On d 4, 3.0 mL of ice cold PBS (0.01 M; pH 7.0) was added to all tubes except the total count tubes. The samples and reagents were then centrifuged at 3000 x G for 1 h at 4°C. Once centrifugation was complete, the tubes were decanted, and the supernatant was discarded. The tubes were then counted in a gamma counter. The intra- and inter-assay coefficients of variation for the controls for LH were 15% and between 5 and 20% (n=2 assays), respectively.

For the statistical analysis, effects of time, treatment, and treatment*time on serum LH concentrations were analyzed. Concentration of P4 were analyzed for comparison during the estrous cycle for the effects of time, treatment, and treatment*time. The data was analyzed by PROC GLM of SAS (SAS; Cary, NC). Data was considered statistically different if $P \le 0.05$.

Ewes were fed 2 lbs per hd per day of a balanced ration for sheep and had ad libitum access to hay and water.

RESULTS

All ewes retained their CIDR for the duration of the protocols except for one ewe in GnRH2, her data was recorded and included. Two ewes and their data that were intended to be included in this study were removed due to issues during blood collection. The Group 2 ewe that lost her CIDR before designated CIDR pull was included in the data.

Mean serum concentrations of LH, shown in Table 1, differed between groups (P < 0.05) from 22- 42 h following CIDR removal. As displayed in Table 1 and Figure 1, the CIDR11 protocol yielded the earliest rise in LH following CIDR removal. The GnRH2, PMSG and CIDR7 groups simultaneously followed with their LH rise about 8 h later. GnRH1 was the last group to show a peak in LH at 30 h.

Table 1. Mean serum concentrations ng/ml of LH for each group; time shown in h after CIDR removal.

Time	GnRH1	GnRH2	PG600	CIDR7	CIDR11	$P > \mathbf{t} $
18	1.64	2.29	3.43	1.80	8.04	0.2216
20	1.83	2.55	3.34	1.87	13.11	0.1096
22	1.70	3.15	4.15	2.20	14.81	0.0681
24	1.85	6.13	4.80	6.09	17.58	0.0137
26	1.74	11.64	10.84	9.10	28.16	0.0001
28	3.74	18.22	15.35	20.26	29.69	0.0001
30	14.46	18.28	14.91	19.21	28.11	0.0001
32	25.16	12.69	14.80	20.57	22.03	0.0001
34	21.11	13.60	12.38	16.11	13.43	0.0001
36	13.70	16.56	7.03	7.01	7.81	0.0006
38	6.81	20.46	4.23	3.06	3.75	0.0089
40	4.01	27.03	4.18	1.90	2.50	0.0070
42	2.88	32.69	3.10	2.06	2.25	0.0037
44	4.61	12.84	2.34	1.33	2.38	0.0979
46	7.60	5.54	2.73	1.33	1.73	0.1796
48	6.09	3.39	2.21	1.20	1.84	0.2936
50	3.25	2.35	2.14	1.06	2.06	0.4370
52	0.06	1.80	2.34	1.51	1.51	0.5084
54	1.76	1.76	1.88	1.29	1.66	0.5494



Figure 1. Mean serum concentrations of LH by treatment from 18 to 54 h following CIDR removal.

The GnRH2 group shows an early rise in LH as a result of 2 ewes that peaked 12 h earlier than the predominant peak for the group. One of those 2 ewes was the individual that lost her CIDR approximately 20 h early. The GnRH2 protocol produced the highest and most defined LH peak at 42 h post CIDR removal (Figure 1). The GnRH1 group showed a defined spike at h 32. The remaining 3 groups showed times of increased serum LH levels, but individual animal peaks are not as harmonious within these 3 treatments. Ewes were observed for signs of estrus from the time of CIDR removal to the end of the bleeding period. Mark times were recorded for each ewe as soon as they were in standing estrus and the ewes had a definite mark from the rams equipped with breeding harnesses. In GnRH2 and CIDR11 all ewes were marked by the rams. The GnRH2 group had the next highest mark rate of 85.71%. The PMSG and CIDR7 treatments followed with 50% and 62.5%, respectively. In GnRH2, 6 of the 8 ewes came in estrus in a 3 h window. One of the 2 ewes that were outside the 3 h window for GnRH2 was the ewe that lost her CIDR approximately 20 h before the set CIDR removal time. The GnRH2 group stands out as the most successful protocol at achieving ovulation during the bleeding period of the trial because all ewes in the treatment ovulated. Treatments for GnRH1 and CIDR11 caused half or more of the ewes to ovulate in a 3 h window (57.14% and 50%, respectively). The CIDR7 group had the narrowest window of ovulation at 6 h, but only 4 of 7 ewes in the group showed an ovulatory spike in LH. The PMSG group resulted in the lowest ovulation rate and the fewest ovulations within a 3 hour window.

DISCUSSION

The goal of this study was to determine which of the protocols being examined is expected to yield the best results when used to synchronize ewes for TAI. Serum LH concentrations from the blood sample data showed treatment to have an effect on serum LH concentrations from 24-42 h following CIDR removal. The GnRH2 protocol resulted in the highest LH concentration at a single time and the most defined peak. Most ewes in this treatment were already beginning to show elevated levels of LH before the second GnRH injection was given at 38 h, but the injection seemed to cause the defined peak that is seen at 42 h post CIDR pull. The LH peaks of ewes in GnRH1 and GnRH2 were farther apart than expected. The protocols are identical out to 38 h after CIDR removal, so it was expected that their peaks would occur at very similar times. Figure 1 shows GnRH2 to have a small rise within 4 h of GnRH1's LH rise. This was due to 2 ewes showing an ovulatory rise in LH at h 28 and 30, but all other ewes in GnRH 2 showed their rise about 8 h later.

The PMSG protocol gave the lowest LH peak of any protocol. This is due to the small number of ewes that ovulated.

The defined LH peaks of GnRH1 and GnRH2 support the idea that these protocols are controlling follicular dynamics at the ovaries. The aspect unique to these two protocols is the intent of the first GnRH injection. It is intended to cause ovulation or atresia of any developing follicles on the ovaries at the present time. Since these two protocols produced the most defined LH spikes, the proposal and intent of the GnRH injection is supported. The second GnRH injection in GnRH2 seemed to produce a higher, longer lasting LH surge in the ewes.

Experiments where GnRH was used to synchronize estrus in cattle have shown similar results. According to Mee et al. (1993), serum LH concentrations were observed to be higher 2 h following a GnRH injection and stayed high for a 6 h period. Data from this study shows LH levels to be rising at 34 and 36 h following CIDR removal, and then a greater rise from 38 to 40 and 42 h. This rise can be attributed to the GnRH injection given at 38 h which caused LH to spike for 4 h before falling. This LH surge from the time of GnRH injection to 4 h following the injection will help to insure ovulation in ewes in this group. It can be assumed that any ewes that had not already ovulated before the injection would ovulate due to the spike following the injection.

The occurrence and time of ovulation was determined to be the time at which serum LH concentration reached 45 ng/ ml. For this experiment, this was assumed to be the ovulatory LH spike. The GnRH2 protocol achieved the highest ovulation percentage of any group. The CIDR11 group followed closely, having only one ewe that didn't show an ovulatory LH peak. The ewe from CIDR11 that did not have an LH spike came in estrus only 5.5 h after CIDR removal. It is possible that she ovulated before the bleeding period began at 18 h. The downfall of this protocol is the wider window of ovulation, especially if one ewe ovulated before the bleeding period began. An aspect added to this protocol to control follicular dynamics may offer a narrower, improved range of ovulation time. The reaction of ewes to the PMSG protocol was less than desirable in terms of the ovulation percentage. These ewes also displayed the widest window of estrus synchrony. This group

Table 2. Number of ewes, percentage of ewes that marked, and percentage of ewes that marked in a 3 h window and range of mark time

Group	n	% Marked	% in 3 h window	Range (h after CIDR removal)
GnRH1	7	85.71	42.86	28-34
GnRH2	8	100.00	75.00	22-39
PG600	8	50.00	25.00	5.5-38
CIDR7	7	62.50	37.50	24-32
CIDR11	8	100.00	50.00	5-34

Table 3. Number of ewes, percentage of ewes that ovulated, percentage of ewes that ovulated in a 3 h window and range of ovulation time.

Group	n	% Ovulated	% in 3 h window	Range (h after CIDR removal)
GnRH1	7	71.43	57.14	30-46
GnRH2	8	100.00	37.50	26-42
PG600	8	37.50	25.00	26-34
CIDR7	7	57.14	28.57	26-32
CIDR11	8	87.50	50.00	20-32

also recorded the lowest ovulation and estrus percentage. One ewe that came in estrus only 5.5 h following CIDR removal this data is supported by the findings of Dickison et al. (2010). Titi et al. (2010) reported that ewes given a long term progestin source and a PG600 injection at progestin removal showed very different times of LH surge.

Data from this experiment supports the idea that a short P4 treatment is more effective than a longer P4 treatment when using other exogenous hormones to synchronizes ewes. The results also infer that a short CIDR coupled with GnRH and PGF2a produces a narrower window of synchrony of ovulation than protocols using PG600 instead of GnRH. This data agrees with findings by Titi et al. (2010) and Jabbour and Evans, (1991).

Several conclusions can be drawn from the data obtained during this project. Primarily, this data demonstrates the potential for a TAI protocol utilizing a short duration P4 implant coupled with one or two injections of GnRH to be a viable synchronization option. In comparison to the standard protocol that is currently being utilized in the industry, the GnRH1 and GnRH2 protocols offer a higher rate of estrus and ovulation and a tighter window of synchrony. Research publications of synchronization protocols for ewes is somewhat limited at this point, but further research in this area will be beneficial to the sheep industry. Comparing the proposed GnRH1 and GnRH2 protocols to the PMSG protocol in an experiment where the ewes are artificially inseminated should be the next step in determining the efficacy of the protocols. A project designed to compare pregnancy rates, lambing rates and number of lambs born per ewe between these protocols will help determine if profit of an operation can be increased through better results from the GnRH protocols. This would also aid the industry in expediting genetic progress because elite stud rams would be able to sire more offspring in their lifetime and after their death.

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Consequence of perennial and annual forage grazing systems before feedlot entry on yearling steer grazing and feedlot performance, carcass measurements, meat evaluation, and system net return

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ABSTRACT: In a 2-year study, yearling steers (n=141), previously wintered for modest gain of <0.454 kg·hd⁻¹·d⁻¹, were randomly assigned in early May each year based on birth date and weight to one of three retained ownership rearing systems: 1) feedlot control (FLT), 2) perennial grass pasture (crested wheatgrass (CWG) > native range (NAT) (PST) or 3) perennial grass pasture followed by annual forage (CWG > NAT > field pea-barley (PBLY) > unharvested corn (CN)) (ANN). During the extended grazing period, grazing annual forages after perennial grasses promoted increased growth (P = <0.0001), rib-eye area (REA, P = <0.0001), fat depth (FD, $P = \langle 0.0001 \rangle$) and percent of intramuscular fat (%IMF, P = 0.0003). At feedlot entry, ANN steers were heavier (P = <0.0001) and required less finishing days on feed (DOF). Compared with the FLT control steers (142 DOF), the number of DOF for the grazing system's steers was 66 and 91 days for the ANN and PST systems, respectively. For feedlot performance, grazing system steer ADG was greater (P =0.006), feed efficiency (FE) better (P = 0.018) and feed cost per unit of gain was lower (P = 0.0005) than for the FLT control steers. Hot carcass weight was heavier for grazing steers (P = <0.0001) than the FLT control; however, no difference was identified for marbling score or percent USDA Choice quality grade. Strip loin steaks (2.54 cm thick) were removed from each carcass half for tenderness, cooking yield, and sensory evaluation. There were no treatment differences for shear force, cooking yield, tenderness, juiciness or flavor. Systems net return was determined without accounting for risk management procedures. The ANN system net return was the most profitable system, returning \$9.09/steer; however, the PST system steers lost -\$30.10/steer, and the FLT control system lost -\$298/steer. These data suggest that retaining ownership through finishing preceded by a long-term sequence of perennial and annual forages improves economically important muscle and fat traits, and the ANN system has the greatest system profit potential.

Key words: feedlot, grazing, meat evaluation, net return, perennial and annual forage, yearling steers

INTRODUCTION

Integrating crop and beef cattle systems may provide a systematic approach to offset normal perennial season forage quality decline (Greenquist et al., 2009) by providing an alternative to declining forage digestibility. Supplementation of yearling heifers grazing northern Great Plains rangeland with distiller's dried grains with solubles for 70 d improved ADG with no adverse effect on feedlot performance or carcass characteristics (Larson et al., 2012). Since yearling and long-yearling cattle make up 45 to 55 percent of total feed lot placements (Brink, 2011), employing a sequence of perennial and annual forages that are systematically grazed in an extended grazing season from May to October (180 d) in western North Dakota may be advantageous for both beef production and cropping systems. Forage quality maintenance, due to sequencing, has potential as a value added enterprise through retained ownership to reduce the number of feedlot days on feed (DOF), while maintaining meat quality, sensory acceptance, and improving net return.

The primary objective of this research was to compare two long-term yearling steer extended grazing systems, prior to feedlot entry, with conventional feedlot growing-finishing to determine the impact on animal performance, days on feed, carcass trait measurements, meat tenderness, sensory panel evaluation and systems net return.

MATERIALS AND METHODS

This research was conducted in western North Dakota at the Dickinson Research Extension Center Ranch Headquarters (14°11' 40"N 102°50'23"W) located 35 km north of Dickinson, North Dakota, USA, in accordance with guidelines approved by the NDSU Institutional Animal Care and Use Committee.

Animals and Experimental Design

After weaning in November of each year (2011 and 2012), medium- to large-frame steers (5-7 frame score; n = 141) were wintered for modest gain of <0.454 kg·hd⁻¹·d⁻¹ grazing corn aftermath plus medium-quality alfalfa-

bromegrass hay (Medicago sativa and Bromus inermis). In early May, the steers were assigned randomly to one of three triple-replicated treatments based on birth date and weight: 1) Feedlot direct control (FLT), 2) Perennial grass pasture (PST), or 3) Perennial grass pasture and seeded annual forage fields (1.74 ha) (ANN). The FLT control steers were shipped directly to the University of Wyoming, Sustainable Agriculture Research Extension Center, Lingle, Wyoming, and fed to final harvest weight. The PST treatment steers grazed crested wheatgrass (Agropryon desertorum (CWG)) followed by native range comprised of the following major plant species: blue gramma (Bouteloua gracilis), western wheatgrass (Pascopyrum smithii), green needlegrass (Nassella viridula), needle and thread (Stipa comate), little bluestem (Schizachyrium scoparium), and prairie sandreed (Calamovilfa longifolia) (NAT). The ANN treatment consisted of a forage sequence of CWG followed by NAT, as previously described, plus 1.74 ha fields of field pea-barley intercrop (Pisum sativum, var. Arvika and Hordeum vulgare, var. Stockford (PBLY)) and unharvested corn (Zea mays (CN)), (e.g. CWG > NAT > PBLY > CN).

At the end of an average 182 d extended grazing period, the PST and ANN forage grazing treatments were transferred to the University of Wyoming feedlot and fed to final harvest.

During the grazing season, PST steers were moved from spring crested wheatgrass to native range pastures in mid-June and, for the ANN treatment, the steers were moved from crested wheatgrass to native range in mid-June and from native range to PBLY the third week of August each year. After PBLY grazing was completed, the steers were moved to standing unharvested corn. Forage crude protein change was determined with bi-monthly sampling from three locations in the PST and ANN treatments.

The design was to graze each forage type until forage crude protein (CP) content declined to a range of 8.0 to 10.0 percent CP or the pasture or field was sufficiently grazed. Grazing season cost per steer for the perennial (CWG and NAT) pastures was determined using a constant cost per kg of body weight of \$0.00198 multiplied by the start weigh and end weight to arrive at a daily grazing cost. Then, using onehalf the total number of days grazed, the first half and second half grazing charges were summed to arrive at the total grazing charge per steer. For the ANN treatment, the grazing cost was based on the sum of the custom grazing charge for the CWG and NAT pastures, plus the actual farming input costs for crop establishment and \$12.15 per ha cash rent for western North Dakota non-irrigated cropland.

The number of feedlot DOF was determined using ultrasound measurements for rib-eye muscle area (longissimus dorsi), external fat depth and percent of intramuscular fat. At the packing plant, carcass data was collected on chilled carcasses after a 48-hour chill. After grading, strip loin steaks were removed from each carcass half between the 12th and 13th ribs and frozen for shear force and sensory panel evaluation (AMSA, 1995) at the NDSU Meats Laboratory.

Statistical analysis

The animal performance data was analyzed using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment and year as fixed effects and performance and carcass measurements as dependent variables. Hot carcass weight was used as a covariate to adjust carcass values. Sensory panel and shear force data were analyzed using HH the GLM procedure of SAS. Pen (pasture) served as the experimental unit. MIXED and GLM least-square means were separated using the predicted difference option of SAS and differences were considered significant at $P \le 0.05$.

RESULTS AND DISCUSSION

Grazing and feedlot performance data have been summarized in Tables 1 and 2. Steer growth rate for the PST and ANN steers was 0.77 and 1.0 kg·hd⁻¹·d⁻¹, respectively, for the average 182-d grazing season, resulting in a total grazing season gain of 140 and 183 kg·hd⁻¹ for the PST and ANN extended grazing system treatments, respectively. The total grazing cost per kg of gain was higher for the ANN treatment (\$1.12 vs. \$1.30 for PST and ANN, respectively).

Grazing annual forages (PBLY > CN) after native range improved economically important muscle and fat measurements prior to feedlot entry. When measured with ultrasound at the end of the grazing season, REA (P =<0.0001), FD (P = <0.0001) and the %IMF (P = 0.0003) were significantly greater for the ANN than the PST systems, which may have contributed to a numerically greater number of ANN steers having carcasses grading Choice or better after the finishing period.

Feedlot performance for either of the extended grazing systems (PST and ANN) was superior to the FLT control steers. The FLT control steers averaged 1.73 kg·hd⁻¹·d⁻¹ and reached slaughter weight earlier than steers in the PST and ANN forage grazing systems; however, once the grazing system steers entered the feedlot, their compensating ADG was significantly greater (P = 0.006) than the FLT control.

FLT control steers were 18.1 months of age at slaughter, compared with 21.4 and 22.1 months of age for the ANN and PST systems, respectively. Although grazing increased the number of days from birth to slaughter, grazing (PST and ANN) dramatically reduced the number of DOF in the feedlot. Compared with the FLT control that averaged 142 DOF, the ANN steers reached final slaughter weight after a short 66 DOF and the PST steers required 91 DOF. This difference in the number of DOF to reach final slaughter weight is a direct result of combining perennial and annual forages in a sequence in which the ANN steers grazed higherquality forage throughout the extended grazing season.

Thus, compared with the ANN treatment, declining late summer and fall native range forage quality resulted in lesser REA, FD and %IMF among the PST system steers. Declining late-season forage quality required the PST steers to be on feed for an additional 25 days to reach the final harvest end point.

Table 1. Effect of grazing system on yearling steer grazing performance

				P-Value			
	PST	ANN	SE	Trt	Yr	Trt x Yr	
No. Steers	48	47					
Grazing:							
Days Grazed	181	183					
Start Wt., kg	369	375	2.54	0.058	< 0.0001	0.76	
End Wt., kg	509 ^a	558 ^b	3.81	< 0.0001	0.004	0.002	
Gain, kg	140 ^a	183 ^b	2.47	< 0.0001	< 0.0001	0.0001	
ADG, kg	0.77 ^a	1.0 ^b	0.14	< 0.0001	< 0.0001	0.0003	
Cost/Head, \$c, d	157.19 ^a	238.36 ^b	0.81	< 0.0001	0.36	0.005	
Cost/Lb Gain, \$	0.5571	0.5924	0.015	0.14	< 0.0001	0.001	
Fat and Muscle:							
REA, sq cm	55.9ª	70.1 ^b	0.71	< 0.0001	0.54	0.01	
FD, cm	0.58	0.84	0.017	< 0.0001	< 0.0001	0.0006	
IMF, %	3.22	4.13	0.11	0.0003	0.047	0.25	

^{a-b}Means within a row with different superscripts differ (P < 0.05).</p>

^c Field Pea-Barley Crop Input Cost – Seed \$25.40/ac, Seeding \$15/ac, Innoculant \$5.08/ac, Pre-Plant Chemical \$3.18/ac, Windrowing \$10/ac, Land Rent \$30/ac = (\$88.66/ac x 13.5 ac)/24 Steers = \$49.87/Steer; Mean Days Grazed: 26 days

^d Unharvested Corn – Seed \$47.82/ac, Planting \$15/ac, Fertilizer (Urea \$37.85/ac, MESZ \$28.69/ac,

Potash \$4.96/ac), Chemical \$3.43/ac, Land Rent \$30/ac = (167.75/ac x 13.5 ac)/24 Steers = \$94.36/Steer; Mean Days Grazed: 52 days

Table 2. S	ystems	feedlot	finishing	performance

						P-Value	
	PST	ANN	FLT	SE	Trt	Yr	Trt x Yr
No. Steers ^d	48	47	46				
Feedlot Days on Feed	91	66	142				
Harvest age, Months	22.1ª	21.4 ^b	18.1 ^c	0.043	< 0.0001	0.0001	0.003
Feedlot Start Wt., kg	487 ^a	539 ^b	367 ^c	6.8	< 0.0001	0.65	0.002
Feedlot End Wt., kg	675 ^a	671 ^a	612 ^b	8.2	0.0002	0.71	0.21
Feedlot Gain, kg	188 ^a	132 ^b	245°	5.5	< 0.0001	0.27	0.014
Feedlot ADG, kg	2.07 ^a	2.00^{a}	1.73 ^b	0.068	0.006	0.33	0.006
Feed:Gain, kg	2.83 ^a	2.79 ^a	3.13 ^b	0.109	0.018	0.19	0.0001
Feed Cost/Head, \$	381.18 ^a	276.12 ^b	578.30°	7.62	< 0.0001	< 0.0001	0.0002
Feed Cost/kg Gain, \$	2.03 ^a	2.09 ^a	2.36 ^b	0.077	0.005	0.003	0.001

a-cMeans within a row with different superscripts differ (P < 0.05).

^dANN: one steer died of bloat after entry into unharvested corn; FLT: one steer bloated and died each year.

Despite reaching the slaughter end point sooner, feedlot performance for the FLT control system steers was inferior in most of the economically important criteria measured. In total and compared with the FLT control, extended grazing systems that delay feedlot entry resulted in better feedlot ADG (P = 0.006), FE (P = 0.018), feed cost per steer (P = < 0.0001) and feed cost per kg of gain (P = 0.005). This does not agree with the findings of others (Larson et al., 2012; Greenquist et al., 2009).

Carcass measurements and meat evaluation criteria are summarized in Table 3. For carcass trait measurements, average HCW for the FLT control system was 78 pounds lighter than the average of the two pasture systems, which is likely due to the fact that steers in the grazing systems' treatments were an average 3.7 months older. Although a numerically smaller number of carcasses graded Choice or better, no statistical difference was found among the systems' treatments for quality grade. Steer carcasses from the PST and ANN forage systems tended to have larger REA (P = 0.078), as well as greater FD (P = 0.033). Marbling score and quality grade did not differ between FLT, PST, and ANN treatments; however, YG was lower (P=0.042) for the FLT steers.

Meat tenderness and sensory panel evaluations of strip loin steaks (Table 3) did not differ among treatments for Warner-Bratzler shear force and cooking meat yield. Sensory panel evaluation of the steaks showed no difference for perceived tenderness, juiciness or flavor.

Table 3. Carcass closeout and quality gr	rade comparison between extended	d grazing and feedlot direct systems
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					P-Value		
	PST	ANN	FLT	SE	Trt	Yr	Trt x Yr
No. Steers	48	47	46				
Hot Carcass Weight	854.5 ^a	850.7 ^a	774.8 ^b	9.30	< 0.0001	0.14	0.032
REA, sq cm	83.9 ^a	80.9 ^b	78.1°		0.078	< 0.0001	0.16
SE ^d	(0.22)	(0.20)	(0.33)				
FD, cm	1.30 ^a	1.27 ^a	0.94 ^b		0.083	0.91	0.001
SE ^d	(0.022)	(0.021)	(0.032)				
Marbling Score ^e	516.0	529.7	501.2		0.58	< 0.0001	0.82
SE ^d	(19.2)	(18.1)	(27.5)				
YG	2.93 ^a	2.82 ^a	2.41 ^b		0.042	< 0.0001	0.0001
SE ^d	(0.083)	(0.077)	(0.123)				
QG Choice or Better, %	82.1	86.5	65.6		0.312	0.017	0.023
SE ^d	(6.15)	(5.70)	(9.46)				
Meat Evaluation:							
Warner-Bratzler Shear Force, kg	3.53	3.15	3.31	0.12	0.11		
Cooking Yield, %	81.0	84.2	82.5	1.04	0.062		
Sensory Tenderness ^f	5.10	5.02	5.54	0.11	0.40		
Sensory Juiciness ^f	5.63	5.53	5.78	0.10	0.26		
Sensory Flavor ^f	5.78	5.87	5.91	0.09	0.25		

^{a-c} Means within a row with different superscripts differ (P < 0.05). ^d SE: hot carcass weight used in covariate analysis

^e Marbling score: 400 = small marbling; 500 = modest marbling

^f1 = extremely tough, dry, bland; 8 = extremely tender, juicy, flavorful

Table 4. Systems income, expense, and net return

	PST	ANN	FLT
No. Steers	48	47	46
Income:			
Gross Carcass Value/Head, \$	1718.41	1738.93	1497.41
Expenses:			
Steer Cost/Head, \$	1041.72	1051.56	1034.02
Wintering Cost/Head, \$	60.00	60.00	60.00
Grazing Cost/Head			
Perennial Grass, \$	157.19	94.13	
Field Pea/Barley, \$		49.87	
Standing Unharvested Corn,\$		94.36	
Feedlot Feeding Cost/Head, \$	381.18	276.12	578.30
Transportation, Health & Brand, \$	108.42	103.80	123.14
Total System Expense/Head, \$	1748.51	1729.84	1795.46
Net Return/Head, \$	-30.10	9.09	-298.05
Sh (1.1.1 1.00)	1100 (D - 0.07)		

^c Means within a row with different superscripts differ (P < 0.05).</p>

The system's two-year average income, expense, and net return are summarized in Table 4. Utilizing annual forage as a way to extend the grazing season 112 d longer, in this study, compared to the 70 d grazing period reported by Larson et al. (2012), reduced the number of feedlot DOF by 54%. The ANN system showed a positive net return of \$9.09 per steer and the PST system lost -\$30.10 per steer. The PST net loss is attributed to slower growth due to declining forage quality during the latter part of the grazing season, which is in agreement with Caton and Dhuyvetter, 1997. The conventional feedlot control system lost -\$298.05; a margin of \$307.14 between the ANN and FLT systems.

IMPLICATIONS

The results of this study indicate that extended grazing systems can reduce the cost of production among steers held for retained ownership. The ANN extended grazing system that included grazing annual forages during the late summer and early fall seasons prior to feedlot entry is a systematic procedure whereby cow-calf producers can capitalize on their herds genetics profitably and do so without risk management intervention.

The decision for cattlemen to use an extended yearling grazing program to capture value added profits will be determined by several factors such as the implications of crop insurance, cost for adequate fencing, reliable water sources, and the estimated return from competing crops or enterprises.

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Effects of weaning method on weaning-and growing-phase performance by early-weaned calves

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ABSTRACT: We evaluated the health and performance of early-weaned steer calves during a 56-d weaning period and a subsequent 56-d feedlot receiving period. Steers (n = 239; 128 ± 14 d of age) were assigned randomly to 1 of 2 weaning treatments: drylot weaning for 56 d (DRYLOT) or pasture weaning for 56 d (PASTURE). Steers assigned to PASTURE were allowed to graze mature, native tallgrass range without supplement. Steers assigned to DRYLOT were maintained in earth-floor pens and fed a complete, concentrate-based diet for a targeted ADG of 1 kg. Body weight after and ADG during the 56-d weaning period were greater ($P \le 0.01$) for DRYLOT than for PASTURE. Incidence of undifferentiated fever during this period tended to be greater (P = 0.10) for DRYLOT steers than for PASTURE steers. Conversely, incidence of conjunctivitis during the weaning phase of our study was greater ($P \le 0.01$) for PASTURE (40.2%) than for DRYLOT (0%). Steers assigned to DRYLOT had greater (P ≤ 0.01) BW at the beginning of the receiving period, on d 28 of the receiving period, and at the end of the receiving period (d 56) than steers assigned to PASTURE. Steers assigned to DRYLOT also had greater (P = 0.02) ADG from d 1 to 28 of the receiving period than steers assigned to PASTURE. Conversely, there was no difference (P = 0.95) in ADG between treatments from d 28 to d 56. Steers assigned to PASTURE had greater DMI ($P \le 0.01$) during the receiving period than steers assigned to DRYLOT. In contrast, G:F was similar (P = 0.18) between treatments. Incidence of undifferentiated fever was not different (P = 0.99) between DRYLOT and PASTURE during the receiving phase of our study; however, incidence of conjunctivitis was greater ($P \le 0.01$) in steers assigned to PASTURE (14.3%) than in steers assigned to DRYLOT (1.6%). Growth and health during a 56-d weaning period and during a 56-d receiving period were improved when steers were weaned in a drylot environment and fed a concentrate-based diet compared to when steers were weaned in a pasture environment.

Keywords: early weaning, pasture, preconditioning

INTRODUCTION

Early weaning during periods of drought is used commonly by cow-calf producers to reduce grazing pressure on pastures (Rasby, 2007). This practice may result in calves having less value at weaning compared to calves weaned at conventional ages (Story et al., 2000). Retained ownership through a short-term backgrounding period can be useful for improving the value of early-weaned calves. Conversely, feeding concentrate-based diets to calves weaned at less than 125 d of age has been associated with excessive fat deposition early in life and decreased carcass weights at harvest compared to feeding concentrate based-diets to calves weaned at greater than 125 d of age (Barker-Neef et al., 2001; Schoonmaker et al., 2002). In addition, Myers et al. (1999a) reported that early-weaned calves consumed more total concentrate during the finishing period than calves weaned at conventional ages, a circumstance which could negatively affect profit margins during times of high grain prices.

Post-weaning growing programs based on either pasture or high-roughage diets fed in confinement are a viable means to reduce the usage of concentrates without negatively affecting returns. Myers et al. (1999b) achieved similar DOF without affecting harvest BW by grazing early-weaned calves for 82 d before placement into a feedlot compared with early-weaned calves fed a high-concentrate diet from weaning to harvest. Additionally, Bailey et al. (2012) weaned calves in drylot or pasture environments for 28 d before feedlot placement. In spite of reduced BW gain during the weaning and receiving periods, pasture-weaned cattle achieved full compensation of BW by harvest with no differences in finishing DMI, DOF, or carcass quality compared to drylot-weaned cattle. Therefore, the objective of our study was to evaluate the performance and health of early-weaned steer calves subject to a 56-d weaning period in either a pasture or a drylot environment.

MATERIALS AND METHODS

Animal care practices used in our study were approved by the Kansas State University Animal Care and Use Committee (protocol no. 2978.1).

Animals. Angus x Hereford steers originating from the commercial cow-calf herds of Kansas State University (n = 123; initial BW = 132 ± 26.4 kg; 113 ± 13 d of age; Source 1) in Manhattan, KS and the Western Kansas Agricultural Research Center (n = 116; initial BW = 194 ± 23.4 kg; 144 ± 15 d of age; Source 2) in Hays, KS were used in this study. All steers were castrated and dehorned and vaccinated against clostridial diseases (Ultrabac[®] 7; Pfizer Animal Health, Exton, PA) at approximately 60 d of age. At weaning, steers were stratified by source and assigned randomly to 1 of 2

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weaning treatments: drylot weaning for 56 d (**DRYLOT**) at the Western Kansas Agricultural Research Center or pasture weaning for 56 d (**PASTURE**) on native-tallgrass pastures at the Kansas State University Commercial Cow-Calf Unit.

Weaning Phase. Steers from both sources were weighed individually and 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) as they were separated from dams. Calves were also given an injection of trace minerals (Multimin[®] 90; Multimin USA Inc., Fort Collins, CO), treated for internal and external parasites (Dectomax[®] Injectable; Zoetis Inc., Kalamazoo, MI), and given a growth-promoting implant (Ralgro[®]; Intervet Inc., Merck Animal Health, Summit, NJ). Steers were re-vaccinated 14 d after maternal separation.

After initial processing, all steers were transported via motor carrier for a common shipping duration of 4 h to their designated weaning locations.

Steers from both sources that were assigned to DRYLOT were transported to the Western Kansas Agricultural Research Center feedlot, where they were stratified by source assigned randomly to 1 of 8 pens. Pens (minimum area = $200 \text{ m}^2/\text{calf}$; bunk space = 0.46 m/calf) afforded *ad libitum* access to water via concrete tanks.

Calves were fed a diet formulated to promote a 1-kg ADG at a DMI of 2.5% of BW during the weaning phase of the study (Table 1). Feed was delivered once daily at 0700 h; bunks were evaluated each morning at 0630 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 at the conclusion of the experiment and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, ADF, Ca, P, and S (Table 1). Dry matter intake was estimated by dividing the total feed DM delivered to each pen during the weaning period by the average aggregate BW of all steers in the pen during the weaning period.

Steers from both sources assigned to PASTURE were transported to the Kansas State University Commercial Cow-Calf Unit, where they were stratified by source and assigned randomly to 1 of 8 previously-ungrazed, native-tallgrass pastures (97 ± 40 hectares). Upon arrival, steers were confined to a single earth-floor pen (minimum area = 200 m^2 /calf) and allowed *ad libitum* access to native tallgrass prairie hay (89.2% DM, 9.08% CP) via 2 ring feeders (diameter = 3 m) for 4 d. On the afternoon of d 4, steers were released into assigned pastures. Each pasture provided continual access to surface water and was stocked at 3.2 ha/steer. Additional non-study cattle of similar age and weight were added to pastures to achieve the desired stocking density.

Pasture forage quality was estimated by clipping all plant material from within randomly-placed sampling frames (0.25 m²; n = 2/pasture) at a height of 1 cm on 8/7, 9/4, and 10/2 (Table 2).

Health. Steers assigned to both DRYLOT and PASTURE

Table 1. Composition of the weaning diet

	<u>u</u>
Ingredient composition	% DM
Sorghum silage	13.1
Sorghum grain	57.4
Dried distillers grains	20.1
Soybean meal	5.1
Supplement*	4.3
Nutrient composition	DM basis
CP, % DM	18.7
NEm [†] , Mcal/kg DM	1.87
NEg [†] , Mcal∕kg DM	1.15

* Supplement contained ammonium sulfate, limestone, urea, salt, Rumensin 90[®] (300 mg head⁻¹· d⁻¹), Tylan 40[®] (90 mg head⁻¹· d⁻¹), and a trace-mineral premix.
[†] Calculated using NRC (2000) equations.

	Table 2.	Nutrient	composition	of ra	ange f	forage
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Sampling	CP,	NDF,	ADF,
Date	% DM	% DM	% DM
08/07/2013	6.7	60.6	41.0
09/04/2013	6.1	61.1	40.3
10/02/2013	4.8	66.3	46.3

were monitored daily for symptoms of respiratory disease and conjunctivitis. Steers with clinical signs of BRD, as judged by animal caretakers, were removed from pens or pastures and evaluated. Steers were assigned a clinical-illness score (scale: 1 to 4; 1 = normal, 4 = moribund), weighed, and assessed for febrile response. Steers with a clinical illness score > 1 and a rectal temperature > 40.0°C were treated with therapeutic antibiotics according to label directions (first incidence = Baytril[®], Bayer Animal Health, Shawnee Mission, KS; second incidence = Resflor Gold[®], Merck Animal Health, Summit, NJ). Steers were evaluated 72 h following treatment and re-treated if clinical signs of BRD persisted.

Steers showing signs of conjunctivitis (i.e., pinkeye) were treated using oxytetracycline (LA 200[®]; Zoetis Inc., Kalamazoo, MI). Steers were evaluated 14 d following treatment and re-treated if clinical signs of disease persisted.

Receiving Phase. Following the 56-d weaning period, all steers were weighed at their respective weaning sites, implanted with Revalor IS[®] (Intervet Inc.; Merck Animal Health, Summit, NJ), and transported via motor carrier for 4 h to the Western Kansas Agricultural Research Center for a 56-d feedlot receiving period. At that time, steer calves assigned to PASTURE were stratified by source and assigned randomly to 1 of 8 pens, adjacent to those assigned to DRYLOT (minimum area = 200 m²/calf; bunk space = 0.46 m/calf).

To establish a common gut-fill between treatments, all steers were fed the weaning diet (Table 1) at a pre-determined percentage of aggregate pen BW for 7 d. Steers were

Table 3. Composition of the receiving diet

Ingredient composition	% DM
Sorghum silage	13.1
Sorghum grain	57.5
Dried distillers grains	25.9
Supplement*	3.5
Nutrient composition	DM basis
CP, % DM	17.6
NEm [†] , Mcal/kg DM	1.92
NEg [†] , Mcal/kg DM	1.19

^{*} Supplement contained ammonium sulfate, limestone, urea, salt, Rumensin 90[®] (300 mg head⁻¹· d⁻¹), Tylan 40[®] (90 mg head⁻¹· d⁻¹), and a trace-mineral premix.

⁷Calculated using NRC (2000) equations.

weighed on d 7; this BW was used as both the ending BW of the weaning phase of the study and the initial BW of the receiving phase of the study.

Thereafter, all steers were fed a common growing diet once daily at 0700 h (Table 3). Bunks and feed delivery were managed according to procedures described for the DRYLOT treatment during the weaning phase of the study. Bunk samples were collected and analyzed as during the weaning phase of the study (Table 3). Dry matter intake was estimated by dividing the total feed DM delivered to each pen during the receiving period by average aggregate BW of all steers in the pen during the receiving period.

Cattle health was monitored as during the weaning phase of the study. Steers were weighed individually on d 28 and d 56 of the receiving period.

Statistical Analysis. Growth performance was analyzed as a mixed model with a 1-way treatment structure in a completely-randomized design (PROC MIXED; SAS Inst. Inc., Cary, NC). Pen or pasture was the experimental unit. Class factors included treatment, pen or pasture, and source. The model statement included terms for the fixed effects of treatment, source, and treatment \times source. The random statement had terms for pen (or pasture) within treatment and source \times pen (or pasture) within treatment.

Data describing DMI and G:F were analyzed as a mixed model with a 1-way treatment structure in a completelyrandomized design (PROC MIXED; SAS Inst. Inc., Cary, NC). Pen was the experimental unit. Class factors included treatment and pen. The model statement included a term for the fixed effect of treatment only. The random statement was pen within treatment.

Health data were analyzed as a mixed model with a 1-way treatment structure in a completely-randomized design (PROC GLIMMIX; SAS Inst. Inc., Cary, NC). Pen or pasture was the experimental unit. Class factors included treatment, pen or pasture, and source. The model statement included terms for the fixed effects of treatment, source, and treatment \times source. The random statement had terms for pen (or pasture) within treatment and source \times pen (or pasture)

within treatment.

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 $P \le 0.05$. Tendencies were discussed when $0.05 < P \le 0.10$.

RESULTS AND DISCUSSION

Weaning Performance. Body weight after and ADG during the 56-d weaning period were greater ($P \le 0.01$) for DRYLOT than for PASTURE (Table 4). Based on the quality of the forage available to PASTURE steers, these results were expected. Bailey et al. (2012) reported greater BW gains for calves weaned in a drylot environment for 28 d than for calves weaned on dormant native range for 28 d. Conversely, these researchers reported full compensation of BW during the subsequent finishing period with no differences in DOF between pasture- and drylot-weaned calves.

Incidence of undifferentiated fever during the weaning phase of our study tended to be greater (P = 0.10) in DRYLOT steers than in PASTURE steers (Table 4). Similarly, Walker et al. (2007) reported increased morbidity in drylot-weaned calves compared with pasture-weaned calves. Since identical vaccination and health protocols were applied to both treatments in our study, a high occurrence of respiratory disease was not expected from either treatment. Step et al. (2008) indicated that preconditioned calves were less susceptible to disease during the post-weaning period than calves sold through auction markets immediately following separation from dams.

Incidence of conjunctivitis (i.e., pinkeye) during the weaning phase of our study was greater ($P \le 0.01$) for PASTURE steers (40.2%) than for DRYLOT steers (0%; Table 4). This was anticipated, as it was assumed that pasture conditions (e.g., abundant, mature forage) presented a greater risk for corneal lesions than drylot conditions.

Receiving Performance. Steers assigned to DRYLOT had greater ($P \le 0.01$) BW at the beginning of the receiving period, on d 28 of the receiving period, and at the end of the receiving period (d 56; Table 5) than steers assigned to PASTURE. Steers assigned to DRYLOT also had greater (P = 0.02) ADG from d 1 to 28 than steers assigned to PASTURE. Conversely, there was no difference (P = 0.95) in ADG between treatments from d 28 to d 56. Bailey et al. (2012) also reported that pasture-weaned calves had lesser ADG during the first 30 d of the receiving period. In contrast to our results, Bailey et al. (2012) observed that ADG of pasture-weaned calves was less than that of drylot-weaned calves from d 30 to 60 of the receiving period.

Steers assigned to PASTURE had greater DMI ($P \le 0.01$; expressed as a percentage of BW) during the receiving period than steers assigned to DRYLOT (Table 5). In contrast, G:F was similar (P=0.18) between treatments. Bailey et al. (2012) reported greater DMI and G:F by drylot-weaned calves than by pasture-weaned calves during receiving; however, those researchers did not express intake as a proportion of BW. Given the differences in initial receiving BW between drylot-

manageu în pasture or ur	ylot wearing er	ivii onments		
Item	DRYLOT*	PASTURE [†]	SEM	P-value
Weaning BW, kg	163.6	162.8	2.30	0.82
Final BW, kg	222.6	165.5	2.61	< 0.01
ADG, kg	0.93	0.04	0.019	< 0.01
DMI, % BW/d	2.22	-	-	-
G:F	0.247	-	-	-
Undifferentiated fever, %	6.7	0.0	2.71	0.10
Conjunctivitis, %	0.0	40.2	3.17	< 0.01

Table 4. Post-weaning growth and health performance of early-weaned steers managed in pasture or drylot weaning environments

* Steer calves were weaned in a drylot environment and fed a concentrate-based diet for 56 d (8 pens; 14 or 15 steers/pen)

[†] Steer calves were weaned in a pasture environment and not supplemented for 56 d (8 pastures; 14 or 15 steers/pasture)

Table 5. Feedlot-receiving growth and health performance of early-weaned steers managed in pasture or drylot weaning environments

Item	DRYLOT*	PASTURE	SEM	P-value
Initial BW, kg	222.6	165.5	2.61	< 0.01
d 28 BW, kg	257.8	192.6	2.97	< 0.01
d 56 BW, kg	299.9	234.6	3.30	< 0.01
ADG, kg				
d 1 to d 28	1.24	0.97	0.070	0.02
d 28 to d 56	1.51	1.50	0.046	0.95
DMI, % BW/d	2.55	2.63	0.019	< 0.01
G:F	0.177	0.194	0.0082	0.18
Undifferentiated fever, %	2.5	2.5	1.53	0.99
Conjunctivitis, %	1.6	14.3	2.56	< 0.01

Steer calves were weaned in a drylot environment and fed a concentrate-based diet for 56 d (8 pens; 14 or 15 steers/pen)

* Steer calves were weaned in a pasture environment and not supplemented for 56 d (8 pastures; 14 or 15 steers/pasture)

and pasture-weaning treatments, DMI as a percentage of BW may have been similar in both studies.

Incidence of undifferentiated fever was not different (P = 0.99) between DRYLOT and PASTURE during the receiving phase of our trial. Step et al. (2008) indicated that preconditioned, ranch-direct calves were less susceptible to disease during receiving than market-sourced calves with no known health history. Preconditioning management was applied to both of our treatments before feedlot arrival. Incidence of conjunctivitis was greater ($P \le 0.01$) for steers assigned to DASTURE (14.3%) than for steers assigned to DRYLOT (1.6%) during the receiving period. We speculated that there were significant residual effects of the pasture environment on corneal health that lasted well into the receiving period.

IMPLICATIONS

Growth and health during weaning and receiving were improved when steers were weaned in a drylot environment and fed a concentrate-based diet for 56 d compared to when steers were weaned in a pasture environment for 56 d. The drylot-weaned steers in our study were approximately 57 kg heavier at the end of the weaning period and approximately 65 kg heavier at the end of the receiving period than pasture weaned steers. Bailey et al. (2012) reported similar results through the end of the receiving period resulting from 28-d drylot or pasture weaning periods; however, there were no treatment differences in harvest BW, finishing DMI, finishing DOF, or carcass quality. We speculated that the compensatory effects observed by Bailey et al. (2012) during finishing could be sustained for longer periods of time, thus reducing overall feed costs.

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Can method of weaning and subsequent development impact heifer fitness?

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ABSTRACT: Heifer fitness is a collection of traits including behavioral/metabolic adaption and genetic background. Type of weaning and development program implemented sets up the environment replacement heifers must cope to grow and physiologically mature. Objective of this experiment was to determine resting heart rate, average heart rate, time spent loafing and distance traveled as indicators of adaptation to conditions inherent in range or higher input (drylot) heifer weaning and development systems. Spring-born, crossbred heifers were stratified to 1 of 2 treatments at weaning: (1) fence line weaning on native range (NR) with self-fed saltmineral protein supplement (n=118) or (2) weaned into a dry lot (DL) and fed a corn silage diet formulated to gain 0.68 kg/d (n=53). Heifers assigned to DL were separated from their dams and immediately transported to pens at Fort Keogh feedlot while NR heifers were fence line weaned when their dams were removed from a common pasture and moved into an adjacent pasture. A self-fed salt-mineral protein supplement was initiated with NR prior to weaning and 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 (November 2013 to January 2014) a cohort of 21 heifers were fitted with equine heart rate monitors (Polar Equine RS800CX) and QSTARZ CR-Q1100P GPS tracking recorder. Data were recorded until batteries were exhausted (less than 48 hr). Heifers that grazed native range had lower (P < 0.01) loafing (58.9±1.1 vs 68.1± 1.5 beats/min) and average (81.8±3.1 and100.9±3.9 beats/min) heart rates than heifers held in pens. Heifers in the NR system spent a greater (P < 0.01) percentage of time loafing (32.3 ± 1.3) than DL heifers (12.5±1.7%). Distance traveled was influenced due to an interaction (P < 0.01) of month (November, December and January) of measurements and weaning method with NR traveling 3.3, 4.1, 1.7 ± 0.18 (mean = 3.0 ± 0.1) and DL 1.2, 2.6, 2.5 ± 0.27 (mean = 2.1 ± 0.2)km/d in Nov, Dec and Jan respectively. This study revealed that NR heifers preferred to spend more time loafing giving rise to lower average and resting heart rate, suggesting greater fitness and possibly lower energy expenditure, even though the NR heifers traveled further when range was open.

Key words: beef heifers, heifer development, heart rate

INTRODUCTION

Heart rate is an endpoint of energy metabolism. It is the result of oxygen use for aerobic energy utilization to support work of the organism. In the case of muscle and work for activity heart rate should reflect impacts from the environment and metabolic efficiency. A collection of factors influencing metabolic effort and therefore heart rate includes but not restricted to: breed, body weight, temperament (hormone effects), speed, gait, terrain, ground cover, weather variables, experience (coping behaviors), digestive end products, status of activity and individual muscle fiber profile.

Brosh et al. (2007) and Aharoni et al. (2009) implemented heart rate (HR) monitors and GPS recorders along with other measurement 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 made the assumption that "foraging activity increases energy requirements of animals on paddocks compared with confined animals". However there is a scarcity of data utilizing simultaneous HR and GPS recordings to assess the impacts of animals to those systems.

In spring calving herds fall weaning of replacement heifers into a feedlot is widely practiced due to the perceived quality of the environment created by supplying high quality feed, water, and a level of protection from climatic elements compared to the loss of management control when heifers are left to manage for themselves on late season native range. Objective of this experiment was to determine resting heart rate, average heart rate, time spent loafing and distance traveled as indicators of adaptation to conditions inherent in range or higher input (drylot) heifer weaning and development systems.

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 February 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

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weaning heifers grazed alongside their dams in a 350 cow herd, 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 60-70 % occurring during the mid-April through mid-September growing season. Pastures were grazed so that forage was never limiting, water and mineral were always available.

Heifers (n=171) were weaned in the fall and assigned to one of two weaning and development treatments; (1) fence line weaning while grazing native range (NR) with a saltmineral protein (n=118) 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. After weaning, 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 randomly assigned to pens within weight stratification. 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 while the second was hand fed at 1.8 kg hd⁻¹d⁻¹ of a 20% CP starch and fiber supplement. The results of the two supplement strategies will not be reported but the mean of both supplement strategies is reported as a single treatment in comparison to DL. In mid-December due to snow buildup resulting in range forage inaccessibility 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.

Each month (November 2013 to January 2014) a cohort of 21 heifers were fitted with equine heart rate monitors (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 removed 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 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 was inactive (0 distance recorded). Heifers in the drylot 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 loafing was sum of consecutive minutes when there was not a change in GPS position reading. We assumed there needed to be at least 5 accumulated minutes in a period for it to be designated as loafing.

Statistical Analysis

Data were analyzed as a completely randomized design with heifer as the experimental unit (N=63) using the Kenward-Roger degrees of freedom method. The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to test all main effects and all possible interactions. The model included weaning/development management, month, and weaning/ development management × month. Means were separated using least significant. Birth date was used for covariate analysis. All interactions remained in the model regardless of significance. Differences were considered significant when $P \le 0.05$.

RESULTS AND DISCUSSION

Heart Rate

Individual resting heart rate can be interpreted as a general indicator of fitness between animals. There is a inverse relationship between resting HR and the level of fitness. A higher resting HR suggests in most cases more work is required for homeostasis. Resting heart rate was influenced (P<0.01) by an interaction of month and weaning/ development management. The main effects showed DL heifers had higher (P < 0.01) resting HR moving from range to DL. In addition, as the environment becomes colder animals will increase their HR to compensate for the effects of the colder temperature. In November NR and DL treated heifers had similar resting HR. As winter progressed to Dec and Jan the DL heifers (Fig.1) had higher (P < 0.01) resting HR than NR heifers. Heifers in the NR (Fig.2) system in Jan were found to have higher heart rates than in Nov but similar to Dec which was intermediate and not different than Nov or Jan. In Nov and Dec resting HR were similar for NR heifers but higher in Jan. Both groups of heifers had higher resting HR in Jan than Nov but the increase was 12% in NR and 25% in DL (Table 1).

Month and weaning/development management interacted (P < 0.01) to affect heifer average HR. Overall, DL heifers had the highest (P<0.01) average HR in the months of Dec and Jan and were greater (P<0.01) than Nov measurements. In Jan, NR treated heifers experienced the highest average HR than other months for NR treated heifers but they were at least 23 beats/min less than DL in Dec or Jan. The lowest (P < 0.01) average HR was found in the NR



Figure 1. Heart rate for heifer (ID#13426) in dry lot for 24 h starting at 12:00pm on December 16, 2013. Average heart rate = 105, distance traveled 3.15 km.



Figure 2. Heart rate for heifer (ID#13654) on native range for 24 h starting at 12:00 pm on December 16, 2013. Average heart rate = 62, distance traveled 3.83 km.

heifers in Nov and Dec which was similar to the value found for DL heifers in Nov. Moving heifers from range to dry lot created a situation where average and resting heart rates increased indicating a loss of fitness or an increase in stress. The DL heifers tended to be less adaptable to winter weather as shown by a 55% greater change in resting HR from Nov to Jan compared to NR.

Distance Traveled

Travel is a response by cattle grazing on range or exploring a dry lot in response to various drives or urges. The motives driving traveling behavior have been described by Rouda et.al (1990) which include forage quality, feed accessibility, water (quality and quantity), wind and other climatic events, terrain, pasture size and shape, breed and animal irritability among others. It is assumed that cattle with shorter grazing bouts have preferred grazing conditions than animals that must graze longer. Less time is spent grazing when forage is abundant, immature being easier to tear and consume and cattle are understood to be more content. Dairy literature has shown, lying times are lower and standing times higher when dairy cattle are uneasy or less comfortable (Haley et al., 2001).

Month (November, December and January) of measurements interacted (P < 0.01) with weaning/ development management to influence distance traveled in 25 hour time period (Table 1). Heifers assigned to DL (Fig. 3) traveled 2km more (P < 0.05) in Dec and Jan than they did in Nov. Since food seeking behavior is not primary motivator in the dry lot the increased distance traveled maybe associated with less comfort in the pens or potentially indicative of poor adaption to the surroundings. Heifers in NR (Fig 4.) had the opposite response to the advance in season by traveling less in Jan. In the months of Nov and Dec NR heifers were grazing to acquire their diet and consequently traveling longer distances (P<0.01). By mid Dec snowfall accumulation was sufficient to restrict effective grazing so grass hay was fed halting the search and grazing behavior and NR responded by traveling less (P<0.05) in Jan than in Nov or Dec. However they also traveled less than the DL heifers possibly indicating incomplete adaptation by DL with the conditions. This relationship is further supported by the lesser percentage of time spent resting by the DL heifers possibly due to their inability to adjust to the dry lot conditions. They spent 5 to 13% of their time loafing while the NR heifers used 27 to 33% of their time to loaf (Table 1). The DL heifers spent less time idle and in Jan traveled more distance than heifers fed hay on pasture demonstrating potential greater energy expenditure when not eating.

resting and distance	resting and distance durrened in 25 nouts (kin)							
		Location						
		Drylot		Nati	ve Range	_		
Measurement	Nov	Dec	Jan	Nov	Dec	Jan	SEM	P-value
Average Heart Rate	89 bc	121 a	117 a	76 c	74 c	94 b	6	0.02
Resting Heart Rate	60 bc	76 a	75 a	55 c	59 bc	62 b	3	0.04
% Time Resting	13 c	10 cd	5 d	27 b	30 ab	33 a	2	0.01
25 hour Distance (km)	1.2 d	2.6 c	2.5 c	3.3 b	4.0 a	1.8 d	0.2	< 0.0001

Table 1. Effect of location and month on average heart rate (beats/minute), resting heart rate (beats/minute), percent of time resting and distance traveled in 25 hours (km)

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



Figure 3. Tracing of GPS coordinates for heifer in dry lot in December.



Figure 4. Tracing of GPS coordinates for heifer grazing native range in December.

Body Weight and Change

At weaning heifers in each weaning/development management group had similar body weights. However as expected the DL heifers gained over 2 times (P < 0.05) the body weight as the NR heifers (38 vs 15kg for DL and NR).

IMPLICATIONS

Heifers in DL were found to have greater average or resting HR either due to a loss of fitness after leaving the range setting or the pen conditions imposed a higher oxygen demand due to hormonal stimulation due to stress or increased energy expenditure needed to cope with the pen environment. This finding is further exaggerated since the NR heifers in Nov and Dec were grazing and traveled further distances and maintained lower HR. In the conditions of this study fitness and adaptability appear to be components of coping mechanisms used by range heifer calves after weaning.

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Effects of QuietWean nose flap devices applied to calves on cow and calf performance

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ABSTRACT: The objective of this study was to determine if QuietWean nose flap (NF) devices could be used on calves for a short time, followed by returning calves to normal nursing, to effectively increase dam body condition. This study was conducted at 2 locations using primiparous (2-yrold) Angus and Angus \times Hereford cows (n = 245). Cow and calf pairs were allocated to 4 treatments in a completely randomized design. Treatments on the calves were: 1) NF for 30 or 31 d while remaining with their dams and removal from dam to the feedlot on d 30 or 31 (LTNF d 30 R), 2) NF for 4/5 d while remaining with their dams and removal from dam to the feedlot on 30/31 d (STNF d 30 R), 3) NF for 4/5 d while remaining with their dams and removal from dam to the feedlot on 60/62 d (STNF d 60 R), and 4) no NF while remaining with their dams and removal from dam to the feedlot on 30/31 d (Control d 30 R). Body weight from cows in Control d 30 R was less ($P \le 0.03$) than cows in LTNF d 30 R or STNF d 30 R at d 60 or 62. Cows in LTNF d 30 R gained more ($P \le 0.0001$) from d 0 to 120 or 122 than all other treatment groups, while cows in STNF d 60 R gained less ($P \le 0.03$) than all other treatments. Fat thickness as measured by ultrasound did not differ among treatments (P = 0.18). While there was no difference in BCS among d 60 or 62 and 120 or 122, cows in STNF d 60 R decreased ($P \leq$ 0.001) in BCS from d 0 to 60 or 62; whereas all other groups increased in BCS. Calves in LTNF d 30 R gained less ($P \le$ 0.0001) than calves from all other treatments. There was no difference ($P \le 0.05$) in BW between calves in STNF d 30 R and STNF d 60 R; however both treatments gained less than calves in Control d 30 R ($P \le 0.0001$). Calves in Control d 30 R gained more than calves in the other treatments (P \leq 0.0001). These results indicate that NF weaking devices can have an effect on minimizing nursing of calves and can improve performance of cows.

Key words: beef calves, BCS, fat thickness, nose flap, twostage weaning

INTRODUCTION

Typical weaning methods generally include sudden separation of calves from their dams. Removing calves from cows decreases the energy requirements of the cows and allows them to begin improving in body condition. As pregnancy rates are higher in cows maintaining moderate BCS or rising towards moderate (Houghton et al., 1990), it is important to allow adequate time for cows to reach proper condition in order to conceive another pregnancy. Cow BCS increases and pregnancy rates improve as calf age at weaning decreases (Myers et al., 1999). However, it can be useful to continue the use of milk from the dam as a beneficial supplement to growing calves. Therefore, it is important to equally consider both cow and calf gains to optimize their performance in the weaning process. Several methods have been studied to observe their effects on cow and calf performance after weaning (Price et al., 2003, Enriquez et al., 2009). One method has been the use of nose flap (NF) weaning devices. A NF can inhibit the nursing of calves while continuing to allow them to consume feed and water.

The objective of this study was to examine whether NF devices could be used for a short period of time followed by returning calves to normal nursing to effectively allow an increase in body condition while allowing calves to remain on pasture with their dams to achieve added growth with no supplementation.

MATERIALS AND METHODS

This experiment was conducted following Colorado State University Animal Care and Use Committee approval. Two pasture locations were used for this study, each containing herds consisting of 153 (location 1) and 92 (location 2) primiparous Angus and Angus x Hereford cows. Body weight and BCS of cows were collected on d 0, 60 or 62, and 120 or 122. Fat thickness (cm) was measured by ultrasound on d 0 and 60 or 62. Calves were weighed on d 0, and 30 or 31, and 60 or 62.

Experimental Design and Treatments

The experiment was a completely random design. Cows were diagnosed for pregnancy and production records were evaluated before the study. Non-pregnant cows and cows with incomplete records were removed from the study. Remaining cow/calf pairs were then randomly assigned to 1 of 4 treatments at each location: 1) NF for 30- or 31-d (longterm; LT) while remaining with dam followed by removal from dam to a feedlot on d 30 or 31 (LTNF d 30 R), 2) NF for 4- or 5-d (short-term; ST) while remaining with dam followed by removal (R) from dam to a feedlot on d 30 or 31 (STNF d 30 R), 3) NF for 4- or 5- d while remaining with dam followed by removal from dam to a feedlot on d 60 or 62 (STNF d 60 R), and 4) no NF while remaining with dam followed by removal from dam to a feedlot on d 30 or 31 (Control d 30 R). As seen in Figure 1, calves in LTNF d 30 R, STNF d 30 R, and STNF d 60



Figure 1. Schedule of nose flap weaning treatments applied to calves¹

 ^{1}ST = short-term, LT = long-term, NF = nose flaps, R = removal from dam.

R treatment groups were fitted with QuietWean (JDA Livestock Innovations Ltd, Saskatchewan, Canada) NF and returned to their dams on d 0. On d 4 or -5, depending on location, calves were again gathered and NF were removed from the STNF d 30 R and STNF d 60 R groups. On d 30 or 31, cows and calves were gathered and NF from LTNF d 30 R group were removed. Calves from the LTNF d 30 R, STNF d 30 R, and Control d 30 R were then separated from dam and placed in a feedlot. The STNF d 60 R group remained on pasture with their dams until d 60 or 62.

Statistical Analyses

Data were analyzed as a completely random design using the PROC Mixed procedure of SAS (v. 9.2; SAS Inst. Inc., Cary, NY) to produce a mixed model, with cow or calf as the experimental unit. There was one location × treatment interaction ($P \le 0.02$) for calf BW, so data for calf BW were analyzed by location. There were no location × treatment interactions ($P \le 0.05$) for BCS, BW, or fat thickness variables, so data were pooled across locations. Means were separated using the LSMEANS procedure of SAS using least significant differences when $P \le 0.05$.

RESULTS AND DISCUSSION

Cow Performance

There was no location × treatment interaction for cow BW (P = 0.27), BCS (P = 0.18), or fat thickness (P = 0.50). On d 60 or 62, Control d 30 R cows weighed less ($P \le 0.03$) than LTNF d 30 R and STNF d 30 R cows (Table 1). Cows from the LTNF d 30 R treatment gained more from d 0 to 60 or 62 ($P \le 0.02$) and 0 to 120 or 122 ($P \le 0.0001$) than all other treatment groups. There was no difference (P = 0.85) in gain between cows from the STNF d 30 R and Control d 30 R on d 0 to 120 or 122. Cows from the STNF d 60 R gained less ($P \le 0.03$) than all other treatment groups. It is important to note that there was no difference in STNF d 60 R cows and Control d 30 R cows at d 60 or 62, as the former treatment included nursing calves for an additional 30 d than the latter.

There was no difference (P = 0.51) in ultrasound fat thickness ultrasound among treatment groups at d 60 or 62 (Table 2). All cows decreased in fat thickness from d 0 to 60 or 62 (P = 0.34)

There was no difference in BCS at the beginning of the study (P = 0.39). Body condition score was different on d 60 or 62 ($P \le 0.01$) among treatment groups (Table 3). Cows from STNF d 60 R had a lower BCS ($P \le 0.01$) than all other treatments. This c an be attributed to the change in BCS. Cows

Table	1.	Effect	of	weaning	treatment	applied	to ca	lves or	cow	BW
					er werenn witte	apprice				** **

		Trea	_			
	LTNF	STNF	STNF	Control	-	
Item	d 30 R	d 30 R	d 60 R	d 30 R	SEM	P - Value
BW, kg						
d 0	497.5	510.5	505.9	501.1	5.7	0.40
d 60/62	516.6 ^a	517.7 ^a	502.3 ^{ab}	498.1 ^b	5.7	0.03
d 120/122	535.5	535.0	523.2	525.8	5.7	0.24
BW gain or loss, kg						
Δ Wt d 0 to 60/62	19.3 ^a	7.1 ^b	0.4 ^{bc}	-3.2°	3.8	< 0.001
Δ Wt d 60/62 to 120/122	18.8 ^{ab}	18.6 ^{ab}	16.5 ^a	27.7 ^b	3.4	0.09
Δ Wt d 0 to 120/122	38.3 ^a	25.9 ^b	16.9 ^c	25.2 ^b	2.7	< 0.0001

^TLTNF d 30 R = nose flaps (NF) for 30- or 31-d while remaining with dam, calves removed (**R**) from dam on d 30or 31; STNF d 30 R = NF for 4- or 5-d while remaining with dam, calves removed from dam on d 30 or 31; STNF d 60 R = NF for 4- or 5-d while remaining with dam, calves removed from dam on d 60 or 62; Control d 30 R = no NF while remaining with dam, removed from dam on d 30 or 31.

^{a-c}Means within a row without common superscripts differ ($P \le 0.05$).

There was no location × treatment interaction ($P \ge 0.27$)

	LTNF	STNF	STNF	Control	-	
Item	d 30 R	d 30 R	d 60 R	d 30 R	SEM	P - Value
Fat Thickness, cm						
d 0	0.51	0.44	0.47	0.52	0.03	0.18
d 60 or 62	0.33	0.35	0.30	0.36	0.03	0.51
Fat gain or loss, cm Δ fat d 0 to 60 to						
	-0.19	-0.09	-0.18	-0.17	0.04	0.34

 Table 2. Effect of weaning treatment applied to calves on cow subcutaneous fat thickness as measured by ultrasound

¹LTNF d 30 R = nose flaps (NF) for 30- or 31-d while remaining with dam, calves removed (**R**) from dam on d 30or 31; STNF d 30 R = NF for 4- or 5-d while remaining with dam, calves removed from dam on d 30 or 31; STNF d 60 R = NF for 4- or 5-d while remaining with dam, calves removed from dam on d 60 or 62; Control d 30 R = no NF while remaining with dam, removed from dam on d 30 or 31. Control d 30 R = no NF while remaining with dam, removed from dam on d 30/31.

There was no location \times treatment interaction (P > 0.05).

Table 3. Effect of weaning treatment applied to calves on cow BCS1

		Treat				
Item	LTNF	STNF	STNF	Control	SEM	P - Value
	d 30 R	d 30 R	d 60 R	d 30 R		
BCS						
d 0	5.43	5.55	5.48	5.48	0.07	0.39
d 60/62	5.59 ^a	5.65 ^a	5.42 ^b	5.62 ^a	0.08	< 0.01
d 120/122	5.51 ^{ab}	5.57 ^a	5.40 ^b	5.52 ^{ab}	0.08	0.10
BCS gain or loss						
Δ BCS d 0 to 60/62	0.16^{a}	0.10^{a}	-0.07 ^b	0.14 ^a	0.04	< 0.001
Δ BCS d 60/62 to 120/122	-0.07	-0.09	-0.03	-0.10	0.05	0.77
Δ BCS d 0 to 120/122	0.09 ^a	-0.002^{a}	-0.09 ^b	0.04 ^a	0.05	0.08

¹BCS scale is 1 = thin, 9 = obese (Wagner et al., 1988).

²LTNF d 30 R = nose flaps (NF) for 30/31-d while remaining with dam, calves removed (**R**) from dam on d 30/31; STNF d 30 R = NF for 4- or 5-d while remaining with dam, calves removed from dam on d 30/31; STNF d 60 R = NF for 4- or 5-d while remaining with dam, calves removed from dam on d 60/62; Control d 30 R = no NF while remaining with dam, removed from dam on d 30/31.

^{a-b}Means within a row without common superscripts differ ($P \le 0.05$).

There was no location \times treatment interaction (P > 0.05).

from STNF d 60 R treatment group continued to decrease in BCS ($P \le 0.0001$) while all other treatment groups increased in BCS. This trend was not as significant in d 60 or 62 to 120 or 122 ($P \le 0.77$) as the calves had all been weaned, however total change in BCS in STNF d 60 R remained lower ($P \le 0.02$) than all other treatments.

These results support previous findings that weaning calves earlier result in improved cow performance (Houghton et al., 1990; Myers et al., 1999; Story et al., 2000,). When calves were left in the pasture with their dams for 60 or 62 d, cow BW and BCS decreased. However these findings do not support a similar study where calves were fitted with NF for 14 d and no change in BW or BCS was observed (Quintans., et al, 2009). This could be due to an environmental effect of the type and quality of pasture available to the cows.

Calf Performance

There was a location × treatment interaction ($P \le 0.02$) for calf BW. This is likely due to a difference in the forage quality available to the calves in the pasture, however forage quality was not monitored in the current study. As seen in Table 4, the average BW of calves in location 2 began lower than location 1, so it was expected that there would be differences in the results.

There was no difference in calf weights on d 0 and 30 or 31 at location 1. Calves in Control d 30 R gained more ($P \le 0.0001$) than all other treatment groups. There was no difference in gain among calves in STNF d 30 R or STNF d 60 R. Subsequently, LTNF d 30 R calves gained less ($P \le 0.01$) than calves in all other treatments.
		Treat	ment ¹			
	LTNF	STNF	STNF	Control		
Item	d 30 R	d 30 R	d 60 R	d 30 R	SEM	P - Value
Location 1						
BW, kg						
d 0	227.1	221.7	222.9	218.5	4.1	0.54
d 30 or 31	247.1	246.2	247.4	250.1	4.2	0.92
d 60 or 62	281.4 ^a	279.8 ^a	259.0 ^b	281.3ª	4.2	0.0002
BW gain or loss, kg Δ calf BW d 0 to 30 or						
31	20.1 ^a	24.5 ^b	24.0 ^b	31.6°	1.3	< 0.0001
Δ calf BW d 0 to 60 or 62	54.1ª	58.1 ^{ac}	36.1 ^b	62.7 ^c	2.2	< 0.0001
Location 2 ²						
BW, kg						
d 0	196.2	202.1	201.7	201.1	5.2	0.84
d 30 or 31	212.7 ^a	227.2 ^{ab}	226.2 ^{ab}	228.0 ^b	5.3	0.13
d 60 or 62	242.2 ^a	262.7 ^a	221.3 ^b	263.1 ^a	3.4	< 0.0001
BW gain or loss, kg Δ calf BW d 0 to 30 or						
31	16.1 ^a	24.3 ^b	24.5 ^b	26.9 ^b	1.8	< 0.001
Δ calf BW d 0 to 60 or	5 4 1 B	50.186	ac th	(0.55		.0.001
62	54.1	58.1	36.1	62.7	4.9	< 0.001

Table 4. Effect of weaning treatment applied to calves on calf BW by location²

¹LTNF d 30 R = nose flaps (NF) for 30/31-d while remaining with dam, calves removed (**R**) from dam on d 30/31; STNF d 30 R = NF for 4- or 5-d while remaining with dam, calves removed from dam on d 30/31; STNF d 60 R = NF for 4- or 5-d while remaining with dam, calves removed from dam on d 60/62; Control d 30 R = no NF while remaining with dam, removed from dam on d 30/31.

^{a-c}Means within a row without common superscripts differ ($P \le 0.05$).

²Because of a location × treatment interaction ($P \le 0.02$), calf weight data is being reported by location.

At location 2, Control d 30 R calves weighed more ($P \le 0.0001$) than LTNF d 30 R calves on day 60 (location 1) or 62 (location 2). These results were expected, as the NF prevented nursing from those dams. Once removed from their dams and moved to the feedlot, there were no differences among LTNF d 30 R, STNF d 30 R, or Control d 30 R calves. For both locations, calf BW at d 60 or 62 and change in calf BW on d 0 to 60 or 62 was lower in calves from STNF d 60 R as the other calves entered the feedlot and were fed a higher quality ration. These findings support those reported by Haley et al. (2005), that the use of NF weaning devices lowered calf ADG during implementation of the treatment, but ADG was not affected once calves were removed from their dams.

IMPLICATIONS

Through implementation of nose flaps for a 4 or 5, or 30 or 31 d period, cow and calf performance data indicate that a similar protocol could minimize nursing of calves while maintaining ADG and improve weight gain of cows. The data presented is preliminary data and additional data such as

rebreeding rates will need to be evaluated to fully determine the effects of the nose flap protocols in the present study on cow performance. Additional research is needed to further examine the use of nose flaps as a weaning protocol and the effect of cow and calf performance.

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Effects of ingestion and collection bag type on nutrient composition of masticate samples collected from esophageally fistulated cattle

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ABSTRACT: Ten esophageally fistulated cattle fitted with either screen (SCR; N=5) or solid (SOL; N=5) bottom collection bags were presented with 410g (DM) grass hay (GHAY; 7.1% CP, 80% NDF), 170g (DM) fresh vegetative grass (MFR;15.1% CP, 56% NDF) harvested immediately before presentation, 416g (DM) alfalfa hay (AHAY; 19.5% CP, 49% NDF) and 109g (DM) fresh vegetative alfalfa (AFR; 19.1% CP, 40% NDF) harvested immediately before presentation. Masticate samples were weighed to calculate percentage of forage offered recovered in the collection bag. All masticate and pre-ingested forage samples were immediately frozen and stored until lyophilized and analyzed for CP, NDF, ash and IVOMD. Ingestion status (pre-ingested (PRE) vs. post-ingested (POST)) affected levels of ash (P <0.001). Crude protein levels were generally higher for PRE vs. POST (P < 0.1) but were similar for GHAY (P > 0.1). Levels of NDF were similar for PRE vs. POST (P > 0.1)except for AFR P < 0.1). In general, IVOMD was not affected by ingestion status (P > 0.1), except for GHAY (P < 0.1). Bag (SCR vs. SOL) did not affect ash and NDF (P > 0.1) except for AFR (P < 0.03). Bag did not affect CP of alfalfa (P = 0.71) but did affect grass CP (P = 0.02). Digestibility was not affected by bag (P > 0.1). Forage type (FRESH vs. HAY) influenced the amount of the diet that was recovered through the esophageal opening (P = 0.01). In general, masticate samples were lower (P < 0.01) in CP and similar in NDF and IVOMD (P > 0.1) compared to pre-ingested forage. Ash levels were higher (P < 0.1) in masticate than pre-ingested forage due to the minerals added by saliva.

Key words: esophageal diet collection, grazed diets, sample preparation

INTRODUCTION

Fistulated animals have been used extensively to quantify nutrient intake of grazing animals. This method accounts for the grazing animal's selectivity which is not accounted for in clipped samples. Several factors inherent to using fistulated cattle may affect the degree to which forage masticate samples actually represent grazed animal diets. Changes in chemical composition of forage collected by this method have been attributed to mastication followed by salivary contamination and leaching (Acosta and Kothmann, 1978). Salivary contamination and sample preparation technique could influence both the organic and inorganic components of grazed grass samples (Hoehne, et al., 1967). Collection bags with screen bottoms have long been used (Edlefsen et al., 1960; Barth and Kazzal, 1971; Scales et al., 1974) and allow for drainage of excess saliva which facilitates shorter sample drying times. Nutrients leach from the forage into the saliva and are lost with the loss of the saliva from the bag. Forages of different quality may be affected to differing degrees. Previous research (Musgrave, et al, 2012) has shown a higher loss of nutrients for vegetative forage compared with hay or dormant forage. Therefore, the objectives of this study were to compare the nutrient composition of forage fed to cattle with that of masticate samples collected through esophageal fistula and to determine the influence of collection bag type (screen vs solid) on the nutrient composition of vegetative or dry alfalfa or meadow grass masticate samples collected from esophageally fistulated cattle.

MATERIALS AND METHODS

On days 1 and 4, 10 esophageally fistulated cattle were held off feed for 12 h, then the esophageal plug was removed. Cattle were then fitted with either solid (SOL; N=5) or screen (SCR; N=5) bottom collection bags. On d 1, cattle were presented with 410g (DM) grass hay (7.1% CP, 80% NDF) and allowed to completely consume it (15-20 min). Masticate was removed from the bag and cattle were then offered 170g (DM) vegetative meadow grass (15.1% CP, 56% NDF) harvested immediately before presentation. On d 4, cows were offered 416g (DM) alfalfa hay (19.5% CP, 49% NDF) and allowed to completely consume it (15-20 min). Masticate was removed from the bag and cattle were then offered 109g (DM) fresh alfalfa (19.1% CP, 40% NDF) harvested immediately before presentation. Pre-ingested forage was randomly sub-sampled for chemical analysis. Amount of each forage offered was chosen to ensure the forage would be completely consumed by the animal. No orts remained in the feed pan for any forage. Masticate samples were collected and weighed to calculate percentage of forage offered that was recovered in the collection bag. All masticate and preingested forage samples were immediately frozen and stored until lyophilized. Samples were analyzed for nitrogen using a Leco, FP 2000 combustion nitrogen analyzer (Leco Corp, St. Joseph, MO) then converted to CP by multiplying by 6.25. Neutral detergent fiber content was determined using the Van Soest et el. (1991) method and IVOMD using the Tilley and Terry (1963) method. Values for CP and NDF were expressed on an OM basis.

Data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC) as a 2 x 2 factorial arrangement of treatments in a completely randomized design. The model included ingestion status (pre vs post), forage type (fresh vs. hay), and bag type (screen vs solid) as fixed effects and cow as a random effect.

RESULTS

Ingestion status (pre-ingested (PRE) vs. post-ingested (POST)) affected levels of ash (10.1% vs. 15.0% ash for PRE vs. POST, respectively; P < 0.001, Table 1). The higher ash content POST is in agreement with results reported by Barth and Kazzal (1971), Bath, et al. (1956), and Hoehne et al. (1967). The post ingestion increase in ash content of forage samples may be adjusted for by expressing the other chemical components on an organic matter basis. The addition of minerals by the saliva make samples collected through the esophageal fistula unacceptable for determination of mineral composition of the forage.

Crude protein levels were generally higher for PRE vs. POST (P < 0.1, Table 1) but were similar for GHAY (7.6% vs. 7.8% CP for PRE vs. POST, respectively; P > 0.1). This is in agreement with previous research (Musgrave et al., 2012) which reported a larger difference in CP between pre and post ingested samples of higher quality than for lower quality forage samples.

Levels of NDF were similar for PRE vs. POST (P > 0.1, Table 1) except for AFR (43.9% vs. 49.9% NDF for PRE vs. POST respectively; P < 0.1). Musgrave et al. (2012) reported an increase in NDF of higher quality forages while lower quality forages remained unchanged. Cell solubles from fresh vegetative grass may go into solution more rapidly than those of the dry hay, possibly accounting for some of the difference observed.

In general, IVOMD was not affected by ingestion status (P > 0.1, Table 1), except for GHAY (55.7% vs. 61.1% IVOMD for PRE vs. POST, respectively; P < 0.1). Barth and Kazzal (1971), reported a decrease (P < 0.05) in IVDMD for tall fescue while IVDMD for orchardgrass was similar (P > 0.05) for pre and post ingested samples (-2.8% vs -1.0% difference in IVDMD from pre to post ingested samples for tall fescue vs orchardgrass, respectively).

Bag (SCR vs. SOL) did not affect ash and NDF (P > 0.1, Table 2) except for AFR (14.5 % vs. 20.8% ash; P=0.02 and 47.4% vs. 53.1% NDF; P=0.03 for SCR vs. SOL, respectively). Bag did not affect CP of alfalfa (P = 0.71) but did affect grass CP (11.5% vs. 11.1% CP for SCR vs. SOL, respectively; P =0.02). Digestibility was not affected by bag (67.3% vs. 67.6% IVOMD for SOL vs. SCR, respectively; P > 0.1).

Forage type (FRESH vs. HAY) influenced the amount of the diet that was recovered through the esophageal opening (70.5% vs. 52.8% OM for FRESH vs. HAY, respectively; P =0.01, Table 3). Barth and Kazzal (1971), found DM recoveries from fescue and orchardgrass pastures to be similar to FRESH values (66% DM FRESH vs. 67% and 68% DM for fescue and orchardgrass, respectively).

Masticate samples of high quality forage were lower (P < 0.01) in CP whereas lower quality forage masticate samples were similar (P > 0.1) to pre-ingested forage values, which agrees with the findings of Musgrave et al., (2012). Masticate NDF and IVOMD were similar (P > 0.1) to pre-ingested forage. Ash levels were higher (P < 0.1) in masticate than pre-ingested forage, likely due to the minerals added in the saliva. Lower recoveries suggest masticate samples may not always be representative.

	Fre	esh	H	ay			P-values	
	Pre	Post	Pre	Post	SE^1	Type ²	Ingest ³	$T \ge I^4$
Alfalfa								
Ash, %DM	9.4°	17.4^{a}	10.6 ^c	14.0 ^b	0.9	0.21	< 0.001	0.01
CP, %OM	21.1 ^a	19.3 ^b	21.8 ^a	19.8 ^b	0.5	0.18	< 0.001	0.85
NDF, %OM	43.9°	49.9 ^b	55.3ª	52.7 ^{ab}	1.5	< 0.001	0.17	0.002
IVOMD, %	68.3 ^a	68.5 ^a	62.0 ^b	63.4 ^b	1.0	< 0.001	0.44	0.61
Meadow								
Ash, %DM	13.2 ^b	18.0^{a}	7.1 ^d	10.4 ^c	0.8	< 0.001	< 0.001	0.37
CP, %OM	17.5 ^a	14.8 ^b	7.6°	7.8°	0.2	< 0.001	< 0.001	< 0.001
NDF, %OM	64.8 ^b	62.8 ^b	86.1 ^a	83.3 ^a	1.6	< 0.001	< 0.14	0.81
IVOMD, %	$77.8^{\rm a}$	76.8 ^a	55.7°	61.1 ^b	0.9	< 0.001	0.004	< 0.001

Table 1. Nutrient composition of pre- and post-ingested fresh or dry alfalfa or grass

Standard error of the simple effect mean.

²Main effect of forage harvest status.

³Main effect of forage ingestion status.

⁴Forage harvest status by ingestion status interaction.

	Fre	esh	H	ay		J	P-values	
	SCR	SOL	SCR	SOL	SE^1	Type ²	Bag ³	$T \ge B^4$
Alfalfa								
Ash, %DM	14.5 ^b	20.8 ^a	13.5 ^b	14.5 ^b	1.3	0.04	0.02	0.07
CP, %OM	19.4	19.2	19.9	19.7	0.7	0.44	0.71	0.99
NDF, %OM	47.4 ^b	53.1 ^a	52.8 ^a	52.7 ^a	2.4	0.05	0.03	0.40
IVOMD, %	70.0 ^a	66.5 ^{ab}	63.1 ^b	63.7 ^b	1.9	0.02	0.37	0.34
Meadow								
Ash, %DM	18.3 ^a	17.6 ^a	9.7 ^b	11.1 ^b	1.5	0.001	0.81	0.51
CP, %OM	15.0 ^a	14.6 ^a	8.0 ^b	7.6 ^b	0.2	< 0.001	0.02	0.88
NDF, %OM	64.3 ^b	61.2 ^b	83.7 ^a	82.8 ^a	2.9	< 0.001	0.39	0.76
IVOMD, %	77.6 ^a	76.2 ^a	59.5 ^b	62.6 ^b	1.6	< 0.001	0.48	0.25

Table 2. Nutrient composition of fresh or dry alfalfa or grass masticate samples collected in screen (SCR) or solid (SOL) bottom bags from esophageally fistulated cattle

Standard error of the simple effect mean.

²Main effect of forage harvest status.

³Main effect of collection bag.

⁴Forage harvest status by collection bag interaction.

|--|

	Fre	sh	Ha	iv			P-values		
	Alfalfa	Grass	Alfalfa	Grass	SE^1	Type ²	Forage ³	$T \ge F^4$	
Recovery, % DM Recovery, % OM	68.2 ^a 74.5 ^a	63.8 ^{ab} 66.4 ^{ab}	53.1 ^{ab} 55.1 ^b	48.8 ^b 50.4 ^b	0.1 0.1	0.01 0.01	0.43 0.31	0.99 0.79	

Standard error of the simple effect mean.

²Main effect of harvest status.

³Main effect of forage.

⁴Harvest status by forage interaction.

IMPLICATIONS

These data suggest that forage masticate samples collected through the esophageal fistula may under estimate the amount of CP present in high quality forages but be similar to CP levels in mid- or low quality forages. Masticate samples appear to adequately represent the levels of NDF and IVOMD of forages sampled. Due to increased levels of ash, all values should be reported on an OM basis.

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Influence of supplement composition on utilization of low-quality cool-season forages by beef cattle

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ABSTRACT: Two studies were conducted to evaluate the influence of supplement composition on intake and digestibility of a low-quality (< 6% CP), cool-season forage, as well as cow performance. Treatments included a non-supplemented control (CON), corn (approximately 8% CP), corn and urea (LU = corn + 0.09 mg/kg BW urea, approximately 27% CP; HU = corn + 0.17 mg/kg BW urea, approximately 43% CP) and a positive control of SBM (approximately 51% CP). In Experiment 1, 5 runinally cannulated Angus x Hereford steers (560 \pm 79 kg of BW) were used in an incomplete 5 x 4 Latin square with four 28-d periods. Forage DMI and digestibility were not influenced by supplementation (P > 0.10); however, forage DMI was greater for SBM than HU (P = 0.01). Ruminal NH3-N increased with supplementation (P < 0.01), increased linearly with urea inclusion (P < 0.01), and was greater for HU than SBM (P < 0.01) 0.01). Interestingly, ruminal NH3-N for non-supplemented steers was 1.61 mM, within the range believed to support optimal growth of rumen microbes in vivo, suggesting that ruminally available-N was not limiting forage utilization. Total VFA concentration was not influenced by supplement composition (P > 0.10). In Experiment 2, 80 late-gestation (approximately 190 d pregnant) Angus x Hereford cows (507 \pm 10 kg) were stratified by age, BCS, and BW and randomly allotted to the treatments previously described (20 pens; 4 cows/pen; 4 pens/treatment). Cow BCS change was improved with supplementation (P < 0.01) and with increasing urea inclusion (P < 0.01), but did not differ between the HU and SBM treatments (P > 0.10). Cow insulin, glucose and NEFA were not influenced by supplementation ($P \ge 0.07$) while supplementation increased IGF-I (P < 0.01). These data suggest that a corn-urea based supplement can be utilized as effectively as SBM by ruminants consuming low-quality, cool-season forages as long as the 2 supplements provide comparable intake of CP and energy.

Key words: cattle, cool-season, energy, forage, protein, supplementation

INTRODUCTION

Low-quality forages are a vital part of beef cattle diets; nevertheless, forage utilization is typically limited without supplementation (DelCurto et al., 1990a,b; Köster et al., 1996), leading to reduced BW and BCS (DelCurto et al., 1990b; Bohnert et al., 2002b). This impaired nutritional status and animal performance often leads to reduced reproductive efficiency (Wiltbank et al., 1962; Bellows and Short, 1978; Hess et al., 2005) when compared with an adequate nutritional state. Consequently, many studies have tried to optimize low-quality forage utilization while maintaining animal performance. Protein supplementation typically increases intake and digestibility of low-quality, warm-season forages (DelCurto et al., 1990a,b; Köster et al., 1996); whereas, starch-based supplementation at greater than 0.5% of BW typically decreases forage utilization (Bowman and Sanson, 1996; Garcés-Yépez et al., 1997).

Studies with low-quality, cool-season forages have suggested that forage DMI may not be increased with protein supplementation (Mathis et al., 2000; Bohnert et al., 2002b; 2011). Cool-season forages have a greater proportion of CP as RDP than warm-season forages (Bohnert et al., 2011), suggesting that ruminal NH3-N may not limit intake and digestibility to the same extent as with warm-season forages. Consequently, protein supplementation likely does not have the same positive impact on overall energy intake as seen with warm-season forages. Also, little data is available on the effects of supplementing low-quality, cool-season forages with energy-dense supplements containing varying protein concentrations on ruminant performance and forage utilization.

We hypothesize that energy supplementation will be more beneficial than protein supplementation for ruminants consuming low-quality, cool-season forages. Therefore, the objectives of these experiments were to evaluate the influence of supplement composition on intake and digestibility of low-quality cool-season forages, as well as cow performance.

MATERIALS AND METHODS

Experiment 1. Influence of Supplement Composition on Forage Intake and Digestibility in Steers

Five ruminally cannulated steers (560 ± 79 kg of BW) were used in an incomplete 5 x 4 Latin square and housed in individual pens within an enclosed barn with continuous lighting. Treatments consisted of a non-supplemented, negative control (CON), 3 high energy corn-based supplements with low, moderate and high levels of protein (Corn = 1.26 g/kg BW Corn; LU = Corn + 0.09 g/kg BW urea; HU = Corn + 0.17 g/kg BW urea) and a

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positive control (1.26 g/kg BW SBM). All supplements were formulated to provide similar caloric intakes and the SBM treatment was formulated to provide approximately 100% of the estimated RDP requirement assuming a microbial efficiency of 10% (NRC, 1996; Model 1). In addition, the HU supplement was formulated to be iso-nitrogenous to the SBM supplement; however, a lower than anticipated CP concentration in the corn resulted in the HU supplement having a lower CP concentration than the SBM supplement. The LU supplement was designed to have a CP concentration halfway between that of the Corn and HU supplements. Supplement CP and TDN concentrations (DM basis) were 8, 27, 43, and 51% and 88, 82, 77, and 80%, respectively, for Corn, LU, HU, and SBM. Supplements and a mineral-salt mix were placed directly into the rumen via ruminal cannula at 0700 h daily. Steers had continuous access to fresh water and chopped (4- to 8-cm) fine fescue grass seed straw (4.7%) CP: DM basis). Straw was provided at 0710 h daily at 120% of the previous 5 d average intake; previous day feed refusals were determined prior to supplementation.

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. Treatment effects on ruminal DM and indigestible ADF (IADF) were determined on d 21 by manually removing the contents of the reticulorumen from each steer 4 h after feeding. Total ruminal contents were weighed, mixed by hand and subsampled in triplicate. The remaining ruminal contents were immediately replaced into the animal. Straw, corn and SBM were collected on d 21 through 26 and orts were collected on an equal-weight basis (5% as-fed) on d 22 through 27. On d 23 to 28 fecal grab samples were collected every 12 h with a 2 h advancement each day to allow for sampling on each even hour of a 24-h day. Feed samples were composited by period, whereas orts and feces were composited by steer within period.

Ground samples of feed, orts, and feces were analyzed for DM, OM, and N. Straw, orts and feces were analyzed for NDF and ADF using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.); NDF and ADF of corn and SBM were determined by a commercial laboratory (Dairy One; Ithaca, NY). Feed, orts, feces and rumen particulate samples were analyzed for IADF using procedures described by Bohnert et al. (2002c). Diet digestibility was determined by using IADF fecal concentration in conjunction with DMI and nutrient concentration of forage and supplements (Merchen, 1988).

Blood samples were collected into commercial blood collection tubes containing 0.1 mL of a 15% EDTA solution (Tyco Healthcare Group, Mansfield, MA) via coccygeal venipuncture 4 h after feeding on d 23 through d 28 for analysis of plasma urea N (BUN). Samples were immediately placed on ice for transport to the lab, centrifuged (2,500 x g for 30 min; 4°C) and plasma harvested and stored (-80°C).

Steers were intra-ruminally pulse-dosed with 5 g of Co-EDTA in a 150-mL aqueous solution (Uden et al., 1980) on d 28. Approximately 100 mL of ruminal fluid was collected by suction strainer immediately before dosing and at 1, 3, 6, 9, 12, 18, and 24 h after dosing. Ruminal fluid pH was measured immediately after collection. Twenty milliliters of ruminal fluid was stored (-20° C) for later analysis of Co concentration and 5 mL was acidified with 1 mL of 25% (wt/ vol) meta-phosphoric acid and stored (-20° C) for subsequent analysis of VFA and NH3-N.

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 nonsupplemented; 2) linear effect of urea; 3) quadratic effect of urea; and 4) HU vs SBM. Ruminal pH, NH3-N, and VFA data 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 Composition on Cow Performance

Eighty late-gestation beef cows ($507 \pm 10 \text{ kg BW}$) were stratified into 4 blocks by age, BCS, and BW and randomly assigned within block to 1 of 5 treatments. Cows were then sorted by treatment, within block, and randomly allotted to 1 of 20 pens (4 cows/pen; 4 pens/treatment). The same treatments as described in Exp. 1 were used. Water and a mineral-salt mix were available free choice. Cows were provided ad libitum access to low-quality (5.0% CP; DM basis) fine fescue grass seed straw. The quantity of SBM supplement provided was calculated to meet 100% of the estimated RDP requirement assuming a microbial efficiency of 10% (NRC, 1996; Model 1), while the Corn, LU and HU supplements were provided in amounts estimated to be isocaloric with the SBM treatment. Corn was offered at 816.5g DM cow⁻¹ \cdot d⁻¹, with 59 and 115.2 g DM cow⁻¹ \cdot d⁻¹ of urea added for the LU and HU treatments, respectively; SBM was offered at 816.5g DM cow⁻¹· d⁻¹. The nutrient content of the supplements was the same as described in Experiment 1.

Straw, corn, and SBM 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 postcalving. 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 vaccutainer for serum harvest) via jugular venipuncture at trial onset, d 49, and within 24 h post-calving.

Plasma glucose concentration was determined using a quantitative colorimetric kit (Pointe Scientific, Inc., Canton, MI). Concentration of IGF-I was determined as described by Moriel et al. (2012). Serum NEFA concentration was determined as described by Pescara et al. (2010). Insulin concentration was determined using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostics, Los Angeles, CA).

Cow performance data was analyzed as a randomized block design using the MIXED procedure of SAS. The model included block, treatment, and treatment x block. Blood samples were analyzed using the REPEATED statement with the MIXED procedure of SAS. Model included block, treatment, day, and all resulting interactions. Values were adjusted covariately to values obtained at trial onset. Cow(pen) and pen(treatment) were used as the repeated variables, the subject was cow(pen) and appropriate covariate structure was determined by the Akaike information criterion; AR1 was used for insulin and NEFA and compound symmetry (CS) was used for glucose and IGF-I. The same contrasts as previously described were used to partition specific treatment effects. If no treatment x time interactions were detected (P > 0.05), treatment means were compared.

RESULTS AND DISCUSSION

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

Intake and Digestibility. Intake of grass seed straw was not increased with supplementation (P > 0.10) but was greater for steers receiving SBM than for HU (P = 0.01; Table 1). As designed, N intake increased with supplementation and increased linearly with increasing urea (P < 0.01). However, a lower than expected corn CP concentration resulted in greater CP intake with SBM supplementation than with HU (P < 0.01), possibly explaining the increased straw DMI with SBM.

Apparent total tract DM and N digestibility increased with supplementation ($P \le 0.05$; Table 1), which agrees with previous research, likely because of the greater digestibility of the supplement when compared to the forage. We noted no differences for SBM vs HU or for urea inclusion (P >0.10) on DM digestibility. This agrees with previous work noting ruminal fiber digestibility is not influenced by protein supplementation (Bohnert et al., 2002b; Currier et al., 2004). Furthermore, low-levels of energy supplementation typically do not alter fiber digestibility (Bowman and Sanson, 1996; Garcés-Yépez et al., 1997). In contrast, N digestibility was increased with increasing urea inclusion (P < 0.01) while no difference was noted for HU compared with SBM (P = 0.84).

Blood Variables. Steer BUN was increased with supplementation (P < 0.01; Table 1) agreeing with past research showing increased BUN in response to protein supplementation of low quality forages (Bohnert et al., 2002a) and is directly correlated to N intake. Also, BUN increased linearly with

increasing levels of supplemental urea (P < 0.01) and was greater for HU steers than SBM steers (P < 0.01).

Ruminal Fermentation. Ruminal NH3-N increased with supplementation (P < 0.01), increased linearly with urea inclusion (P < 0.01) and was greater for HU compared with SBM (P < 0.01; Table 1). Non-supplemented steers had a ruminal NH3-N concentration of 1.61 mM, which is within the range of 1.18 to 2.94 mM believed to support optimal growth of rumen microbes in vivo (Slyter et al., 1979). Consequently, it is possible that NH3-N was not limiting ruminal fermentation, and forage DMI, in non-supplemented controls.

Ruminal pH tended to decrease with supplementation (P = 0.08; Table 1) and was lower for HU than SBM (P = 0.01). However, ruminal pH remained above 6.4 for all treatments and sampling times (data not shown). This is well within the range typically considered to support growth of cellulolytic bacteria and fiber digestion, assuming other nutrients are available in adequate amounts (Yokoyama and Johnson, 1988).

No treatment effects were seen on total VFA concentration or molar proportions of propionate and butyrate (P > 0.05; Table 1). Additionally, the acetate:propionate ratio did not differ between treatments (P > 0.10), suggesting similar efficiencies of ruminal fermentation. Nevertheless, the molar proportion of acetate was greater for HU than for SBM steers (P = 0.01) while steers supplemented with SBM had greater molar proportions of the branch chain VFA isobutyrate, isovalerate and valerate ($P \le 0.01$). This was expected, as branch-chain VFA are formed by the fermentation of branchchain amino acids present in natural proteins such as SBM Supplemented steers had greater molar (Leng, 1973). proportions of isovalerate than non-supplemented steers (P <0.01). Isobutyrate tended to decrease linearly with increasing urea inclusion (P = 0.06).

Exp.2 Influence of Supplement Composition on Cow Performance

Pre-calving (within 14 d of calving) BCS change was improved with supplementation (P < 0.01; Table 1) and increased linearly with increasing urea supplementation (P < 0.01). Likewise, post-calving BCS change was increased with supplementation (P < 0.01) and increased linearly with greater urea inclusion (P < 0.01). Although results from Exp. 1 suggests that DMI may have differed between HU and SBM treatments, no differences were noted in pre- or post-calving BCS change for HU compared to SBM cows (P > 0.10). Also, calf birth weight increased linearly (P = 0.04; data not shown) with increasing urea; no incidences of dystocia were noted.

Plasma IGF-I increased with supplementation (P < 0.01; Table 1) and responded in a quadratic fashion to increasing urea (P = 0.05), with IGF-I appearing to plateau when supplemental protein reached the level corresponding to the LU supplement. Despite differences in animal performance between treatments, no treatment effects were detected for plasma insulin, glucose, or serum NEFA concentration (P \geq 0.07; data not shown).

Digestion Study Econom DMI								Contraction of the second seco	S', F =	
Digestion Study Exercise DMI		Τ	reatmenta				Con vs	Г	0	HU vs
Digestion Study	Con	Corn	ΓΩ	ΗU	SBM	SEM^b	Supp	Urea	Urea	SBM
Eorgon DMI										
r utage LIMII,	21.5	20.8	21.7	20.5	23.0	0.71	0.87	0.64	0.10	<0.01
Supplement DMI, g/kg of BW	0.00	1.26	1.35	1.43	1.27					
Total DMI, g/kg of BW	21.5	22.1	23.0	22.0	24.3	0.71	0.02	0.85	0.10	<0.01
N Intake, g/kg of BW	0.165	0.181	0.229	0.258	0.280	0.0064	<0.01	<0.01	0.13	<0.01
DM Digestibility, %	33.9	37.0	37.7	36.5	36.9	1.250	0.05	0.76	0.54	0.79
N Digestibility, %	17.3	20.0	33.3	44.2	43.2	3.584	<0.01	<0.01	0.79	0.84
Blood urea N, mg/dL	10.2	10.2	17.4	22.8	18.5	0.94	<0.01	<0.01	0.35	<0.01
Ruminal NH ₃ -N, mM	1.61	1.50	3.20	4.72	2.96	0.213	<0.01	<0.01	0.69	<0.01
Ruminal pH	6.88	6.81	6.81	6.76	6.88	0.048	0.08	0.38	0.61	0.01
Ruminal Total VFA, mM	134.3	136.4	134.2	135.5	128.0	6.73	16.0	0.93	0.84	0.44
Acetate, mol/100 mol	63.85	63.41	63.22	63.66	61.07	0.672	0.19	0.80	0.71	0.01
Propionate, mol/100 mol	18.06	17.51	18.07	17.99	18.11	0.381	0.63	0.22	0.33	0.75
Isobutyrate, mol/100 mol	1.88	1.85	1.72	1.64	2.38	0.094	0.86	0.06	0.81	<0.01
Butyrate, mol/100 mol	10.86	11.79	11.35	11.48	10.01	0.316	0.15	0.48	0.47	0.22
Isovalerate, mol/100 mol	1.92	2.08	2.25	1.93	3.54	0.204	<0.01	0.51	0.21	<0.01
Valerate, mol/100 mol	3.35	3.22	3.52	3.40	3.94	0.140	0.28	0.36	0.23	0.01
Acetate:propionate ratio	3.56	3.66	3.52	3.58	3.39	0.111	0.84	0.54	0.39	0.16
Cow Performance Study										
Initial BCS	4.76	4.75	4.82	4.62	4.79	0.103	0.86	0.37	0.25	0.21
BCS change										
Precalving	-0.49	-0.32	0.05	0.12	0.26	0.089	<0.01	<0.01	0.17	0.25
Postcalving	-0.63	-0.57	-0.22	-0.05	0.15	0.089	<0.01	<0.01	0.40	0.11
IGF-I, ng/mL	25.0	26.6	39.0	39.4	41.7	2.52	<0.01	<0.01	0.05	0.51
^a Con = control; Corn = corn; LU = low urea; HU ^b $n = 5$	= high urea; SB1	M = soybean	meal.							

IMPLICATIONS

These results suggest that intake of low-quality, coolseason forage was not limited by ruminally available-N. However, the improvement in animal performance with supplementation indicates that both energy and protein were limiting performance. The addition of supplemental energy necessitated the addition of RDP to optimize forage utilization and performance, resulting in similar performance between animals supplemented with natural protein and those receiving an energy dense supplement with added urea. As a result, a starch-based energy supplement, along with a source of NPN, appears to be an acceptable management alternative to sources of natural protein for ruminants consuming lowquality, cool-season forage.

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Effects of stocking rate on forage nutrient composition of Nebraska Sandhills upland range when grazed in early summer

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ABSTRACT: Twelve Nebraska Sandhills upland range paddocks were utilized to determine the effects of stocking rate on grazed forage nutrient composition in early summer. Stocking rates consisted of a con2trol, light, and heavy stocked at 0, 0.54, and 0.82 animal unit months per hectare respectively. Three esophageally fistulated cows per paddock collected diet samples on May 18th (date 1), May 25th (date 2), June 1st (date 3), and June 8th (date 4). Ten quadrats per paddock were clipped and separated into current year growth or previous year growth on each sampling date. Diet and clipped samples were analyzed for CP, NDF, and IVDMD and adjusted to an OM basis. Diet samples had significant treatment x date interactions (P < 0.01) for CP, NDF, and IVOMD. However, treatment and date did not interact (P > 0.05) for clipped samples. Diets collected in control paddocks had greater IVOMD (76.1% vs. 65.1%), (79.8% vs 67.4%), and (78.8% vs. 66.2%) than light on dates 2, 3, and 4, respectively (P < 0.05). Diets collected in control paddocks had greater IVOMD (76.1% vs. 63.2%), (79.8% vs 62.5%), and (78.8% vs. 63.2%) than heavy on dates 2, 3, and 4, respectively (P < 0.05). Diet samples collected from light stocking rate paddocks had greater IVOMD (67.4% vs 62.5%) on date 3 (P < 0.05) than heavy. Diets collected in control paddocks had greater CP (20.5% vs 10.5), (20.5% vs 11.1%), and (18.9% vs. 11.6%) than light on dates 2, 3, and 4, respectively (P < 0.05). Diets collected in control paddocks had greater CP (20.5% vs 8.9%), (20.5% vs 8.8%), and (18.9% vs. 10.8%) than heavy on dates 2, 3, and 4, respectively (P < 0.05). Diets collected in control paddocks had lower NDF (57.9% vs. 78.1%), (45.0% vs. 74.5%), and (42.7% vs. 73.2%) than light on dates 2, 3, and 4, respectively (P < 0.05). Diets collected in control paddocks had lower NDF (57.9% vs. 78.3%), (45.0% vs. 69.9%), and (42.7% vs. 76.7%) than heavy on dates 2, 3, and 4, respectively (P <0.05). Stocking rate affects forage quality and therefore diet quality in early summer.

Key words: Stocking rate, Sandhills upland range, forage quality, sampling method

INDRODUCTION

Upland range in the Nebraska Sandhills is an excellent resource for grazing cattle. Native upland range is dominated by warm-season grass species. Forage quality increases during the spring reaching a peak during June and then steadily declines in quality throughout the remainder of the growing season (Lardy et al., 2004). Research by Lardy et al. has shown changes in forage nutrient composition throughout the year, but effects of stocking rate on Sandhills upland range were not addressed. Cattle are able to select higher quality forage as stocking rate decreases (Heitschmidt and Taylor, 1991). With higher stocking rates, cattle nutrient intake declines. This is in agreement with Kirch et al. (2007) who also stated that cattle are known to be selective grazers and will select a higher quality diet especially as maturation of the plant occurs. With lower quality forage available, cattle may be forced to be less selective. Therefore, the objectives of this research were to determine the effects of stocking rate on forage nutrient quality in early summer pasture and determine if new growth or previous year growth is being consumed in the Nebraska Sandhills.

MATERIALS AND METHODS

Under the approval of the University of Nebraska, Institutional Animal Care and Use Committee, esophageally fistulated cows were utilized to determine the effects of stocking rate on diet quality. Twelve 2-ha upland range paddocks at the Gudmundsen Sandhills Laboratory near Whitman, NE were used. Paddocks were stocked at 0 (control), 0.54 (light) and 0.82 (heavy) animal unit months per hectare with 1st calf heifers varying in wt. Each paddock was continuously grazed and sampled weekly for four weeks in 2013 (May 18th, May 25th, June 1st, and June 8th). Ten 0.25 m² quadrats per paddock were clipped at ground level on each sampling date and separated into previous year growth and current year growth. Additionally, three esophageally fistulated cows were used to sample each paddock on each date to determine diet quality. Esophageally fistulated cows were used for diet sampling and not used in the continuous grazing of the paddocks. Prior to each diet sample collection, cows were withheld from feed, but not water, for 12 h then transported to paddocks where diets were to be collected. Cows were fitted with solid bottom collection bags after removal of the esophageal plug and introduced to the paddock then allowed to graze for about 20 min. After collection esophageally fistulated cows were removed from the paddocks.

Immediately after collection, diet samples were frozen and stored at -20° C, then lyophilized. Clipped samples were dried in a forced air oven at 60° C for 48 h. Both diet and clipped samples were ground to pass a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and analyzed for nitrogen using a Leco, FP 2000 combustion nitrogen analyzer (Leco Corp, St. Joseph, MO) then converted to CP by multiplying by 6.25 and adjusted to an OM basis. NDF content of diet and clipped samples was determined using the Van Soest et al. (1991) method then adjusted to an OM basis. Diet and clipped samples, were analyzed for IVOMD using the Tilley and Terry (1963) method with the modification of adding 1 g of urea to the buffer and ashing the residue to determine OM digestibility. Data were analyzed using repeated measures in PROC GLIMMIX procedure in SAS (SAS Inst., Inc., Cary, NC) with paddock being the experimental unit.

RESULTS AND DISCUSSION

Diet samples had significant treatment x date interactions (P < 0.01) for CP, NDF, and IVOMD. A quadratic effect was observed (P < 0.01, Table 1) for all treatments. However, there were no treatment x date interactions (P > 0.05) in clipped samples. Clipped samples showed a linear decrease in the CP content across all dates for each treatment and a linear increase in IVOMD (P < 0.05) of current year growth across dates for control and light stocking rate paddocks had greater IVOMD (P < 0.05) compared with those collected in light and heavy stocking rate paddocks on collection dates 2, 3, and 4 (Table 3). Diet samples collected in light stocking

rate paddocks had greater IVOMD (P < 0.05) than heavy stocking rate on date 3. Diet samples collected in control stocking rate paddocks had greater CP (P < 0.05) than light and heavy stocking rates on dates 2, 3, and 4. Light and heavy stocking rates showed no difference in CP (P > 0.05) for each sampling date. Diet samples collected from control stocking rate paddocks had lower NDF (P < 0.05) than light and heavy stocking rates on dates 2, 3, and 4. These data suggest that stocking rate has a significant effect on the quality of the diet, helping to explain the treatment x date interaction that was observed. When cattle were introduced into the paddock they were able to select a diet greater in diet quality. As the season progressed, cattle in the stocked paddocks consumed a diet lower in quality than the control paddocks indicating that previous year growth was being consumed. Control stocking rate paddocks did reach peak quality in early June and then decreased in quality which is in agreement with work by Lardy et al. (2004).

For the clipped samples, no differences were shown for previous year growth for CP, NDF, and IVOMD between treatments (P > 0.05) with overall means of 5.2%, 82.0%, and 50.8%, respectively. Current year growth did not differ among treatments for CP, NDF, and IVOMD (P < 0.05) with overall means of 17.4%, 71.7, and 68.7%, respectively. However, CP (P < 0.01) and IVOMD (P < 0.02) content of current year growth increased linearly among treatments. Current growth was greater in CP and IVOMD (P < 0.01, Table 4) than diet sample and previous year growth on all dates. Weir and Torell (1959) observed diet samples were higher in CP than clipped samples. The difference between studies could be due to the separation of current year growth from previous year growth.

Table 1. Nutrient content of diet samples collected from esophageally fistulated cows comparing collection dates by stocking rate

		D	ate						
Item	5/18/2013	5/25/2013	6/1/2013	6/8/2013	SEM	P-value	Linear	Quadratic	Cubic
IVOMD									
Control ²	70.3°	76.1 ^b	79.8 ^a	78.8 ^a	1.27	< 0.01	0.02	< 0.01	< 0.01
Light ³	73.2 ^a	65.1 ^b	67.4°	66.2 ^{bc}	1.27	< 0.01	0.02	< 0.01	< 0.01
Heavy ⁴	71.2 ^a	63.2 ^b	62.5 ^b	63.2 ^b	1.27	< 0.01	0.02	< 0.01	< 0.01
CP									
Control ²	16.2 ^b	20.5 ^a	20.5 ^a	18.9 ^a	1.19	< 0.01	< 0.01	< 0.01	0.08
Light ³	17.1 ^a	10.5 ^b	11.1 ^b	11.6 ^b	1.19	< 0.01	< 0.01	< 0.01	0.08
Heavy ⁴	15.7 ^a	8.9 ^c	8.8°	10.8 ^b	1.19	< 0.01	< 0.01	< 0.01	0.08
NDF									
Control ²	54.4 ^a	57.9 ^a	45.0 ^b	42.7 ^b	3.44	< 0.01	0.99	< 0.01	< 0.01
Light ³	61.2 ^b	78.1 ^a	74.5 ^a	73.2 ^a	3.44	< 0.01	0.99	< 0.01	< 0.01
Heavy ⁴	68.8 ^b	78.3ª	69.9 ^b	76.7ª	3.44	< 0.01	0.99	< 0.01	< 0.01

¹ Standard error of the least squares mean

² Non-stocked paddock (0 AUM/ha)

³ Light stocking rate paddock (0.54 AUM/ha)

⁴ Heavy stocking rate paddock (0.82 AUM/ha)

^{abc} Means within rows lacking common superscript differ (P < 0.05)

		Dat	e		_				
Item	5/18/2013	5/25/2013	6/1/2013	6/8/2013	SEM ¹	P-value	Linear	Quadratic	Cubic
IVOMD									
Control ²	71.5 ^b	77.3ª	73.2 ^b	76.6 ^{ab}	2.74	0.02	0.03	0.30	0.03
Light ³	69.3 ^b	74.5 ^a	75.3 ^a	76.6 ^a	2.74	0.02	0.03	0.30	0.03
Heavy ⁴	72.2 ^a	72.8 ^a	73.2 ^a	74.8 ^a	2.74	0.26	0.03	0.30	0.03
CP									
Control ²	19.2 ^a	17.6 ^a	16.7 ^a	14.0 ^b	1.49	< .01	< 0.01	0.90	0.86
Light ³	19.5 ^a	18.8^{a}	16.4 ^b	16.4 ^b	1.49	< .01	< 0.01	0.90	0.86
Heavy ⁴	19.7 ^a	17.7 ^{ab}	17.0 ^b	15.6 ^b	1.49	< .01	< 0.01	0.90	0.86
NDF									
Control ²	76.1 ^a	71.7 ^a	73.4 ^a	66.4 ^a	4.50	0.53	0.43	0.20	0.74
Light ³	81.1 ^a	86.1 ^a	84.2 ^a	80.3 ^a	4.50	0.53	0.43	0.20	0.74
Heavy ⁴	78.5 ^a	81.6 ^a	80.7 ^a	81.3 ^a	4.50	0.53	0.43	0.20	0.74

Table 2. Nutrient content of clipped samples current year growth comparing collection dates by stocking rate

¹Standard error of the least squares mean ²Non-stocked paddock (0 AUM/ha) ³Light stocking rate paddock (0.54 AUM/ha) ⁴Heavy stocking rate paddock (0.82 AUM/ha) ^{abc} Means within rows lacking common superscript differ (P < 0.05)

stocking rate on each	date				
Item	Control ¹	Light ²	Heavy ³	SEM ⁴	P-value
IVOMD					
5/18/2013	70.3 ^a	73.2 ^a	71.2 ^a	1.88	0.12
5/25/2013	76.1 ^a	65.1 ^b	63.2 ^b	1.88	< 0.01
6/1/2013	79.8 ^a	67.4 ^b	62.5°	1.88	< 0.01
6/8/2013	78.8 ^a	66.2 ^b	63.2 ^b	1.88	< 0.01
CP					
5/18/2013	16.2 ^a	17.1 ^a	15.7 ^a	2.08	0.50
5/25/2013	20.5 ^a	10.5 ^b	8.9 ^b	2.08	< 0.01
6/1/2013	20.5 ^a	11.1 ^b	8.8^{b}	2.08	< 0.01
6/8/2013	18.9 ^a	11.6 ^b	10.8 ^b	2.08	< 0.01
NDF					
5/18/2013	54.4ª	61.2 ^{ab}	68.8 ^b	4.24	< 0.01
5/25/2013	57.9 ^b	78.1 ^a	78.3ª	4.24	< 0.01
6/1/2013	45.0 ^b	74.5 ^a	69.9 ^a	4.24	< 0.01
6/8/2013	42.7 ^b	73.2 ^a	76.7 ^a	4.24	< 0.01

Table 3. Nutrient Content of diet samples collected from esophageally fistulated cows comparing . . . stocking rate on

 6/8/2013 42.7 73.2 76.7

 ¹ Non-stocked paddock (0 AUM/ha)

 ² Light stocking rate paddock (0.54 AUM/ha)

 ³ Heavy stocking rate paddock (0.82 AUM/ha)

 ⁴ Standard error of the least squares mean

 a^{bc} Means within rows lacking common superscript differ (P < 0.05)

Item	Diet1	Live ²	Dead ³	SEM^4	P-value
IVOMD					
5/18/2013	70.4 ^a	71.0^{a}	50.3 ^b	1.07	< 0.01
5/25/2013	66.5 ^b	74.8 ^a	53.2°	1.99	< 0.01
6/1/2013	69.1 ^b	73.9 ^a	49.7 ^c	2.17	< 0.01
6/8/2013	68.4 ^b	76.0 ^a	49.6 ^c	2.38	< 0.01
CP					
5/18/2013	16.1 ^b	21.0^{a}	6.9°	1.12	< 0.01
5/25/2013	13.5 ^b	18.4 ^a	5.4°	1.54	< 0.01
6/1/2013	13.6 ^b	16.8 ^a	5.5°	1.48	< 0.01
6/8/2013	13.8 ^b	15.7 ^a	5.2°	1.64	< 0.01
NDF					
5/18/2013	59.4 ^b	78.6 ^a	82.9 ^a	3.18	< 0.01
5/25/2013	77.4 ^a	79.8 ^a	78.7^{a}	3.06	0.62
6/1/2013	63.2 ^b	79.4 ^a	83.3 ^a	4.81	< 0.01
6/8/2013	64.6°	76.0 ^b	83.0 ^a	6.02	< 0.01

Table 4. Nutrient content of esophageal diet sample versus live and dead clipped samples

¹ Mean diet collection for all treatments using esophageally fistulated cows

² Mean clipped sample for all treatments current year forage growth

³ Mean clipped sample for all treatments for previous year forage growth

⁴ Standard error of the least squares mean

^{abc} Means within rows lacking common superscript differ (P < 0.05)

Neutral detergent fiber was greater (P < 0.01) in clipped samples versus diet samples which was also observed by Weir and Torell. Results indicate that cattle are selective and there are differences between collection methods. Stocking rate affects forage quality and therefore diet quality in early summer. Producers trying to graze upland range earlier in the season need to understand the effects it has on diet quality and manage accordingly.

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Grazing effects on Nebraska Sandhills meadow diet nutrient composition

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ABSTRACT: Twenty-six Nebraska Sandhills sub-irrigated meadow pastures (106 ha \pm 46 ha) were utilized to measure the effects of grazing on forage nutrient composition. Pastures were sampled prior to cattle grazing and after the allotted forage had been grazed. Three esophageally fistulated cows per pasture were utilized for sample collection on thirteen dates spanning from early June through late September. Stocking rates averaged 24, 30, 69, and 46 animal unit days per ha for June, July, August and September, respectively. Samples were analyzed for CP, NDF, and IVDMD and adjusted to an OM basis. Non-grazed pastures exhibited a greater percentage of CP on sampling dates June 17th (14.6% vs. 10.5%, respectively), July 2nd (16.2% vs. 8.03%, respectively), July 11th (10.9% vs. 8.9%, respectively), July 18st (8.8% vs. 7.7%, respectively) July 26th (8.3% vs. 6.5%, respectively), and September 27th (8.97% vs. 6.71%, respectively) than grazed pastures (P < .10). Non-grazed pastures had greater IVOMD on July 15th (68.3% vs. 60.9%, respectively), July 31st (63.7%) vs. 55.7%, respectively), August 8th (65.2% vs. 56.4%, respectively), August 22nd (67.7% vs. 39.8%, respectively), and September 27th, (61.2% vs. 52.3%, respectively) than grazed pastures (P < .10). Non-grazed pastures tended to be higher in IVOMD on June 17th (68.9 vs. 65.4%, respectively; P = 0.12) and August 12th (62.8% vs. 55.2%, respectively; P = 0.11) than grazed pastures. Non-grazed pastures had lower NDF on July 2nd (51.94% vs. 66.4%, respectively; P < .10) than grazed pastures and tended to be lower on June 17th $(55.1\% \text{ vs. } 63.7\%, \text{ respectively; } P = 0.15), \text{ July } 11^{\text{th}} (65.9\%)$ vs. 76.3%, respectively; P = .13) and July 22nd (68.9% vs. 75.3%, respectively; P = .12). No other statistical differences were observed on all other sampling dates for NDF. These data suggest grazing has the most impact on forage quality both early, when the majority of growth occurs, and late in the grazing season when regrowth of cool season grasses occurs.

Key words: grazing, Sandhills meadows, forage quality

INTRODUCTION

The Nebraska Sandhills sub-irrigated meadows are an excellent resource for grazing cattle. Most are dominated by cool-season grass species which have greater growth during early spring. However, as temperatures increase by mid-summer, forage quality decreases (Lardy et al., 2004). Previous research has shown the changes in forage nutrient

composition throughout the year, but it is unclear exactly how grazing affects the nutrient composition of Sandhills sub-irrigated meadows. According to Heitschmidt et al. (1991) stocking rate has an impact on the quantity of the forage consumed and possibly the quality. However, even though stocking rate may have an impact on forage quality maturity is still one of the primary factors affecting forage quality. The decrease in forage quality occurs because of decreasing amounts of in vitro dry matter disappearance and protein content with increasing maturation (Cogswell & Kamstra 1976). Therefore, the objective of this research was to determine the difference in forage quality between grazed pastures vs. non-grazed pastures in the Nebraska Sandhills sub-irrigated meadows.

MATERIALS AND METHODS

Under the approval of the University of Nebraska, Institutional Animal Care and Use Committee, esophageally fistulated cows were utilized to determine the effects of grazing on forage quality. A total of twenty-six sub-irrigated meadow pastures (106 ha \pm 46 ha) in the Nebraska Sandhills were used. The meadow was divided into multiple pastures to allow rotational grazing. Of the twenty-six sampled pastures, two adjacent pastures were sampled on one of thirteen dates throughout the 2013 grazing season: June 17th, June 26th, July 2nd, July 11th, July 15th, July 18th, July 22nd, July 26th, July 31st, August 7th, August 12th, August 22nd, September 6th, or September 27th. Of the two adjacent pastures sampled each date, one pasture was non-grazed while the other pasture had been grazed previously for approximately 4 days. On each sampling date the non-grazed pasture was sampled prior to grazing rotation and the grazed pasture was sampled after rotation to the non-grazed pasture where sampling had just occurred. Stocking rates consisted of 24, 30, 69 and 46 animal unit days per ha for the June, July, August and September samplings respectively. Due to drought concerns, the June and July pastures were stocked lighter than the August and September pastures.

Three esophageally fistulated cows were used to sample each pasture on each date to determine forage quality. Esophageally fistulated animals have been used to collect diet samples as they provide a more accurate estimate of the forage that the animal is consuming (Weir and Torell, 1959). Prior to each diet sample collection, cows were withheld from feed, but not water, for 12 h then transported to pastures where diet samples were to be collected. Cows were fitted with solid bottom bags after removal of the esophageal plug and introduced to the pasture then allowed to graze for about 20 min.

Immediately after collection, diet samples were frozen and stored at -20° C. Samples were lyophilized, ground to pass a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and analyzed for nitrogen using a Leco, FP 2000 combustion nitrogen analyzer (Leco Corp, St. Joseph, MO) then converted to CP by multiplying by 6.25. Neutral detergent fiber content was determined using the Van Soest et al. (1991) method, and IVOMD using the Tilley and Terry (1963) method with the modification of adding 1 g of urea to the buffer and ashing the residue to calculate organic matter digestibility. Data were analyzed using the PROC MIXED procedure in SAS with experimental unit being cow (SAS Inst., Inc., Cary, NC).

RESULTS AND DISCUSSION

Greater CP was observed in non-grazed pastures on June 17th, July 2nd, July 11th, July 18th, July 26th, and September 27th than grazed pastures (P < 0.10; Table 1.). This suggests less difference in protein content during August and early September between grazing and non-grazing. Non-grazed pastures had greater IVOMD on July 15th, July 31st, August 7th, August 22nd, and September 27th than grazed pastures (P

< 0.10). Non-grazed pastures tended to be greater in IVOMD on June 17th (P = 0.12) and August 12th (P = 0.11) than grazed pastures. Non-grazed pastures had lower NDF on July 2nd (P < 0.10) than grazed pastures and tended to be lower on June 17th (P = 0.15), July 11th (P = 0.13), and July 22nd (P = 0.11). No other statistical differences were observed on all other sampling dates for NDF. These data suggest grazing has the most impact on forage quality both early and late in the grazing season when the majority of new growth occurs. In the previous year of this study, similar results were observed in that forage quality was most affected by grazing early in the growing season (Judy et al. 2014).

When cattle are first introduced into a pasture they consume the highest quality forage available. When the highest quality forage becomes less available, the cattle will consume lower quality forage. The lower quality forage could result from consuming more stem or consuming growth from the previous year. Kirch et al. (2007) observed that with lighter stocking rates, cattle are able to be more selective and are not forced to graze as deep into the canopy. During June and July the pastures were understocked due to drought concerns. This could be why significant differences were not observed during all sampling dates in June and July. When higher stocking rates were utilized in August, IVOMD was most affected. However, continued research is needed to determine the effects of grazing on forage quality in subirrigated meadows.

		<u>CP</u>			<u>NDF</u>		<u>1</u>	IVOMD Jngrazed Grazed 68.9 ^a 65.4 ^a 69.2 ^a 66.3 ^a 60.0 ^a 64.1 ^a		
Date ²	Ungrazed	Grazed	SEM ¹	Ungrazed	Grazed	SEM ¹	Ungrazed	Grazed	SEM ¹	
June 17th	14.8 ^a	10.5 ^b	0.93	55.1ª	63.7 ^a	2.63	68.9 ^a	65.4 ^a	0.94	
June 26th	10.2 ^a	9.9 ^a	0.38	67.5 ^a	68.6 ^a	2.23	69.2 ^a	66.3 ^a	1.98	
July 2nd	16.2 ^a	8.0^{b}	1.12	51.9 ^b	66.4 ^a	3.04	60.0^{a}	64.1 ^a	2.93	
July 11th	10.9 ^a	8.9 ^b	0.59	65.9 ^a	76.3 ^a	2.90	62.1 ^a	62.2 ^a	3.03	
July 15th	9.6 ^a	7.8 ^a	0.60	68.4 ^a	73.6 ^a	1.68	68.3ª	60.9 ^b	1.30	
July 18th	8.8 ^a	7.7 ^b	0.39	69.9 ^a	71.6 ^a	2.98	66.3ª	67.0^{a}	1.78	
July 22nd	6.7 ^a	6.5 ^a	0.29	68.9 ^b	75.3 ^a	2.04	64.8 ^a	65.7 ^a	1.49	
July 26th	8.3 ^a	6.5 ^b	0.34	67.4 ^a	67.4 ^a	1.72	66.8 ^a	64.6 ^a	1.85	
July 31st	8.3 ^a	6.4 ^a	0.63	66.5 ^a	75.3 ^a	3.03	63.7 ^a	55.7 ^b	1.70	
August 7th	8.0 ^a	9.1 ^a	0.63	68.9 ^a	66.4 ^a	3.05	65.2 ^a	56.4 ^b	1.74	
August 12nd	7.9 ^a	8.3ª	0.41	64.1ª	67.2 ^a	3.22	62.8 ^a	55.2ª	1.90	
September 6th	8.2 ^a	9.74 ^a	0.60	60.5 ^a	64.7 ^a	3.07	52.3 ^b	61.8 ^a	2.22	
September 27th	9.0 ^a	6.7 ^b	0.45	63.3ª	67.0 ^a	3.16	61.2 ^a	52.3 ^b	1.62	

^{ab} Different subscript between ungrazed and grazed signifies a significant difference with P < 0.10.

1 Standard Error of the least squares mean.

² Date pasture was sampled using esophageally fistulated cattle.

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Supplementation based on protein or energy ingredients to beef cattle consuming low-quality cool-season forages: II. Forage disappearance parameters in rumen-fistulated steers and physiological responses in pregnant heifers

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ABSTRACT: Two experiments evaluated the influence of supplement composition on ruminal forage disappearance parameters, performance, and physiological responses of Angus × Hereford cattle consuming a low-quality coolseason forage (8.7 % CP and 57 % TDN). In Exp. 1, 6 steers fitted with ruminal cannulas were assigned to an incomplete 3 x 2 Latin square design containing 2 periods of 11 d each and the following treatments: 1) supplementation with soybean meal (PROT), 2) supplementation with a mixture of cracked corn, soybean meal, and urea (ENER), or 3) no supplementation (CON). Steers were offered meadow foxtail (Alopecurus pratensis L.) hay for ad libitum consumption. Treatments were provided daily at 0.50 and 0.54 % of shrunk BW/steer for PROT and ENER, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. No treatment effects were detected on rumen disappearance parameters of forage DM ($P \ge 0.33$) and NDF ($P \ge 0.66$). In Exp. 2, 35 pregnant heifers were ranked by initial BW on d -7 of the study, allocated into 12 feedlot pens (4 pens/ treatment), and assigned to the same treatments as in Exp. 1 for 19 d. Treatments were fed once daily at 1.77 and 1.92 kg of DM/heifer for PROT and ENER, respectively. No treatment effects (P = 0.17) were detected on forage DMI. Total DMI was greater (P < 0.01) for PROT and ENER compared with CON. Accordingly, ADG was greater (P =0.01) for PROT compared with CON, tended to be greater for ENER compared with CON (P = 0.08), and similar between ENER and PROT (P = 0.28). Heifers receiving PROT and ENER had greater ($P \le 0.09$) mean concentrations of plasma glucose, insulin, IGF-I, and progesterone compared to CON, whereas ENER and PROT had similar concentrations of these plasma variables ($P \ge 0.15$). A treatment × hour interaction was detected (P < 0.01) for plasma urea N (PUN), given that PUN concentrations increased after supplementation for ENER and PROT (time effect, P < 0.01), but did not change for CON (P = 0.62). In conclusion, beef cattle consuming low-quality cool-season forages had similar ruminal forage disappearance and intake, performance, and physiological status if offered supplements based on soybean meal or corn at 0.5 % of BW.

Keywords: beef cattle, ruminal forage disappearance, lowquality forage, performance, physiology, supplementation

INTRODUCTION

Supplementation is often required in heifer development programs based on low-quality forages. Protein is traditionally considered the limiting nutrient in Western U.S. cow-calf operations (DelCurto et al., 2000), although energy is the primary dietary consideration for female development (Mass, 1987) and forages typically represent the main energy source for forage-fed cattle. Indeed, supplemental protein has been shown to improve digestibility and DMI of lowquality warm0season forages, resulting in increased energy utilization from the forage and cattle performance (DelCurto et al., 1990). However, supplemental protein did not increase forage digestibility and DMI of low-quality cool-season forages Bohnert et al. (2011). Hence, inclusion of energy ingredients into supplements may be beneficial for growth and reproduction of heifers consuming such forages.

After their first breeding season, pregnant heifers still need to grow while maintaining the pregnancy. Energy intake modulates BW gain and circulating concentration of progesterone (\mathbf{P}_{λ}) ; a steroid required for pregnancy establishment and maintenance. The hormones associated with the metabolism of energy substrates, particularly starch, increase P_{4} concentration by reducing hepatic P_{4} catabolism (Cooke et al., 2012) and stimulating ovarian steroidogenesis. Hence, inclusion of energy ingredients into supplements may further benefit reproductive performance of pregnant heifers consuming low-quality cool-season forages by increasing circulating P₄ concentration. However, supplements based on energy ingredients often impair forage digestibility and DMI in cattle (DelCurto et al., 2000). Therefore, 2 experiments compared the effects of supplements based on protein or energy ingredients on ruminal forage disappearance in steers (Exp. 1), and performance and physiological parameters of pregnant beef heifers (Exp. 2).

MATERIALS AND METHODS

Experiment 1

Steers and diets. Six Angus × Hereford steers (initial shrunk BW 494 \pm 11 kg), housed in individual pens (8 × 20 m) and fitted with a ruminal cannula, were assigned to an incomplete 3 x 2 Latin square design containing 2 periods of 11 d each (2 steers/treatment in each period) and the following

treatments: 1) supplementation with soybean [Glycine max (L.) Merr.] meal (PROT), 2) supplementation with a mixture of cracked corn (Zea mays L.), soybean meal, and urea (68:22:10 ratio, DM basis; ENER), or 3) no supplementation (CON). Steers were offered meadow foxtail (Alopecurus pratensis L.) hay for ad libitum consumption during the entire experiment. The PROT and ENER treatments were provided daily at 0.50 and 0.54 % of steer shrunk BW recorded at the beginning of each period, respectively, to ensure that PROT and ENER intakes were isocaloric and isonitrogenous. Urea was included into ENER to result in isocaloric and isonitrogenous intakes of PROT and ENER. Treatment intake during the experiment averaged at 2.20 and 2.37 kg of DM/ steer for PROT and ENER, respectively. Treatments were inserted directly into the ruminal cannula of each steer to ensure readily supplement consumption.

Sampling. Within each period (d 0 to 11), steer shrunk BW was recorded on d0 after 16 h of feed and water restriction to determine steer initial BW. From d 1 to 7 of each period, voluntary forage DMI was recorded daily by collecting and weighing refusals. From d 8 to 11 of each period, steers were offered 90 % of their voluntary forage DMI determined from d 1 to 7. Immediately before treatments were provided on d 8, Dacron bags containing 4 g (DM basis) of ground dietary hay were suspended into the ruminal ventral sac of each steer, and incubated in triplicates for 0, 1, 3, 5, 8, 12, 24, 36, 48, 72, and 96 h. Before ruminal incubation, all bags were soaked in warm water (39°C) for 15 min. After ruminal incubation, bags were washed repeatedly with running water until the rinse water was colorless, and subsequently dried for 96 h at 50°C in a forced-air oven. The 0-h bags were not incubated in the rumen, but were subjected to the same soaking, rinsing, and drying procedures applied to the ruminally incubated bags. Dried samples were weighed for residual DM determination, and triplicates were combined and analyzed for NDF using procedures modified for use in an Ankom 200 Fiber Analyzer.

Statistical analysis. All data were analyzed using steer as the experimental unit and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Kinetic parameters of forage DM and NDF disappearance were estimated using nonlinear regression procedures of SAS, as described by Vendramini et al. (2008). Effective degradability of forage DM and NDF were calculated by fixing ruminal passage rate at 0.046/h (Poore et al., 1990) and using the model proposed by Ørskov and McDonald (1979), whereas treatment effects on these parameters were analyzed using the MIXED procedure of SAS (SAS Inst.). The model statement contained the effects of treatment and period as independent variables. Data were analyzed using steer(treatment \times period) as the random variable. Results are reported as least square means and separated using PDIFF. Significance was set at $P \le 0.05$ and tendencies were denoted if P > 0.05 and ≤ 0.10 .

Experiment 2

Heifers and diets. Thirty-five nulliparous pregnant Angus x Hereford heifers (initial shrunk BW 354 ± 4 kg,

initial age = 508 ± 4 d) were utilized in the study. Heifers were concurrently exposed and became pregnant to a fixed-time AI protocol (CO-Synch + controlled internal progesteronerelease device) 90 d prior to the beginning of the experiment. Pregnancy status to AI was verified by detecting a fetus via transrectal ultrasonography (5.0-MHz transducer; 500V, Aloka, Wallingford, CT) 80 d after AI (d -10). On d -7, all heifers were ranked by initial shrunk (after 16 h of feed and water restriction) BW, and allocated to 12 feedlot pens (4 pens/treatment; 11 pens with 3 heifers and 1 pen with 2 heifers; 8×20 m) in a manner which all pens had equivalent initial average shrunk BW. Pens were randomly assigned to receive the same treatments described in Exp. 1. Heifers were offered meadow foxtail hay for ad libitum consumption during the entire experiment (d -7 to 19). Beginning on d 1, PROT and ENER treatments were fed once daily at a rate of 1.77 and 1.92 kg of DM/heifer, respectively, to achieve the same treatment intake as % of initial shrunk BW used in Exp. 1, and to ensure isocaloric and isonitrogenous intakes. The ENER and PROT treatments were not mixed with hay, and were readily consumed by heifers.

Sampling. Heifer shrunk BW was collected prior to the beginning (d -7) and at the end of the study (d 20; after 16 h of feed and water restriction) for ADG calculation. Hay DMI was evaluated daily from each pen from d 1 to 19 by collecting and weighing refusals daily. Samples of the offered and non-consumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of heifers within each pen, and expressed as kg per heifer/d. In addition, daily intake/heifer of NE_m, NE_g, CP, RDP, and starch were estimated based on total DMI of each pen, and nutritive value of hay and treatments.

Blood samples were collected immediately prior to and 2, 4, 6, and 8 h after treatment feeding (h 0) on d 13, 15, 17, and 19 of the experiment and analyzed for plasma concentrations of glucose, urea N (PUN), insulin, IGF-I, and P₄. Blood samples were also collected on d 0 of the experiment, immediately prior to and 4 and 8 h after hay feeding (h 0) to determine if ENER, PROT, and CON heifers had similar P₄ concentrations prior to the beginning of treatment administration (d 1 to 19). All blood samples were collected via jugular venipuncture into commercial blood collection tubes containing freezedried sodium heparin. After collection, blood samples were placed immediately on ice, subsequently centrifuged (2,500 \times g for 30 min; 4°C) for plasma harvest, and stored at -80°C. Plasma concentrations of P_4 and insulin were determined using Coat-A-Count solid phase 125I RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA). Plasma glucose and PUN were determined using quantitative colorimetric kits (#G7521 and B7551, respectively; Pointe Scientific, Inc., Canton, MI). Concentration of IGF-I was only determined in samples collected at 0 and 4 h after feeding, using a humanspecific commercial ELISA kit (SG100; R&D Systems, Inc., Minneapolis, MN) with 100 % cross-reactivity with bovine IGF-I.

Statistical Analysis. All data were analyzed using the MIXED procedure of SAS, using pen as experimental unit, and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for ADG contained only the effect of treatment. Data were analyzed using pen(treatment) and heifer(pen) as random variables. The model statement used for feed and nutrient intake contained the effects of treatment, day, and the treatment \times day interaction. Data were analyzed using pen(treatment) as the random variable, given that DMI was recorded from each pen. The specified term for the repeated statement was day and subject was pen(treatment). The model statement used for plasma variables contained the effects of treatment, hour, day, and all the resultant interactions. The model statement for P_4 also contained the average P_4 concentration on d 0 as covariate. Data were analyzed using pen(treatment) and heifer(pen) as random variables. The specified term for the repeated statement was hour(day), whereas heifer(treatment \times day) was the subject. For both intake and plasma variables, the covariance structure used was first-order autoregressive, which provided the smallest Akaike Information Criterion and the best fit for the variables analyzed. Results are reported as least square means, or covariately adjusted means for plasma P4 concentration, and separated using PDIFF. Significance was set at $P \le 0.05$ and tendencies were denoted if P > 0.05 and ≤ 0.10 .

RESULTS AND DISCUSSION

Experiment 1

No treatment effects were detected for ruminal disappearance rate or effective ruminal degradability of hay DM ($P \ge 0.33$) and NDF ($P \ge 0.66$; Table 2), indicating that PROT and ENER did not impact rumen in situ disappearance parameters of a low-quality cool-season forage. Supporting these results, Caton and Dhuyvetter (1997) suggested that ruminal disappearance rate of low-quality forages is not impacted by energy or protein-based supplementation. Nevertheless, supplements based on protein and energy ingredients are often associated, respectively, with improved and decreased ruminal forage digestibility in beef cattle (DelCurto et al., 2000). However, protein supplementation is generally beneficial to forage digestibility when the CP content of the basal forage is less than 8 % (DelCurto et al., 2000), whereas the forage utilized herein had 8.7 % CP (DM basis). Supplements based on energy ingredients can be provided to forage-fed cattle at 0.5 % of BW without major impacts on forage digestibility and intake (Bowman and Sanson, 1996), whereas the ENER treatment was provided at 0.54 % of steer BW.

Corn intake above 0.25 % of BW has been shown to impair forage utilization in cattle (Bowman and Sanson, 1996) by reducing ruminal pH, shifting rumen microbes from a cellulolytic population towards an amylolytic population, and decreasing ruminal NH₃ concentration (Caton and Dhuyvetter, 1997). In the present experiment, ENER steers consumed corn at 0.37 % of their BW. However, inclusion

Table 1. Ruminal in situ disappearance parameters of meadow foxtail hay incubated in forage-fed steers receiving no supplementation (**CON**; n = 4), or supplements based on protein (**PROT**; n = 4) or energy ingredients (**ENER**; n = 4)

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Item	CON	PROT	ENER	SEM	<i>P</i> =
Ruminal d	isappearand	e rate, %/h			
DM	2.88	3.36	3.67	0.35	0.33
NDF	3.64	4.24	4.06	0.51	0.71
Effective d	legradabilit	y, %			
DM	60.7	60.8	60.3	1.1	0.95
NDF	55.4	55.5	53.7	1.5	0.66

Table 2. Performance parameters o pregnant beef heifers consuming low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 4), or supplements based on protein (**PROT**; n = 4) or energy ingredients (ENER; n = 4)¹

Item	CON	PROT	ENER	SEM	P =
ADG, kg/d	0.49 ^a	0.89 ^b	0.75 ^b	0.09	0.03
DMI, kg/ d					
Hay	8.60	8.42	8.84	0.14	0.17
Total	8.60 ^a	10.19 ^b	10.50 ^b	0.22	< 0.01
Daily nutrient i	intake				
NE _m , Mcal	9.46°	12.84 ^b	12.89 ^b	0.35	< 0.01
NEg, Mcal	4.73°	7.06 ^b	7.03 ^b	0.22	< 0.01
CP, kg	0.74 ^a	1.62 ^b	1.51 ^b	0.07	< 0.01
RDP. Kg	0.51ª	1.00 ^b	1.12 ^b	0.06	< 0.01
Starch, kg	0.146 ^a	0.239 ^b	0.950 ^b	0.075	< 0.01

¹ Within rows, values with different superscripts differ ($P \le 0.05$).

of a RDP source into corn-based supplements may offset the negative impacts of corn-based supplements on rumen function and digestibility (Olson et al., 1999). Hence, the inclusion of soybean meal and urea into the ENER treatment, as well as the equivalent intake of CP and RDP by ENER and PROT steers, may also have contributed to the similar ruminal forage digestibility among treatments.

Experiment 2

No treatment effects (P = 0.17) were detected on forage DMI (Table 2). This outcome agrees with the lack of treatment effects on ruminal degradability parameters of the forage utilized herein reported in Exp. 1, given that ruminal forage digestibility is positively associated with intake (Allen, 1996). As expected due to the lack of treatment effects on forage intake, as well as treatment design and intake rate, total daily DMI, NE_m, NE_g, CP, and RDP intake were greater (P < 0.01) for PROT and ENER compared with CON heifers, and similar ($P \ge 0.18$) between PROT and ENER heifers (treatment effects, P < 0.01; Table 2). In addition, estimated mean daily intake of starch was greater (P < 0.01) for ENER compared with PROT and CON, and similar (P = 0.40) between PROT and CON (Table 2). Hence, PROT and ENER had a similar increase in energy and protein intake compared with CON heifers, although starch was the main energy source provided by ENER.

A treatment effect (P = 0.03) was detected for ADG (Table 2). In agreement with the treatment effects observed for DMI and nutrient intake, ADG was greater (P = 0.01) for PROT compared with CON, tended to be greater for ENER compared with CON (P = 0.08), and was similar between ENER and PROT (P = 0.28). These results provide evidence that beef heifers consuming low-quality cool-season forages can equally utilize nutrients provided by supplements based on protein or energy ingredients to support BW gain. Supporting this rationale, similar treatment effects were detected for plasma concentrations of PUN (P < 0.01), glucose (P = 0.04), insulin (P < 0.01), and IGF-I (P = 0.03) in the present study (Table 3), which are hormones and metabolites associated with dietary protein and energy metabolism in cattle.

A treatment × hour interaction was detected (P < 0.01) for PUN (Figure 1), given that PUN concentrations increased after supplementation for ENER and PROT heifers (time effect, P < 0.01), but did not change for CON (time effect; P =

Table 3. Plasma concentrations of urea N (PUN), glucose, insulin, IGF-I, and P₄ of pregnant beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 4), or supplements based on protein ingredients (**PROT**; n = 4) or energy ingredients (**ENER**; n = 4).

Item	CON	PROT	ENER	SEM	P =
PUN, mg/dL	4.6 ^a	16.3 ^b	18.5 ^b	1.9	< 0.01
Glucose, mg/dL	62.2ª	66.5 ^b	66.6 ^b	1.3	0.04
Insulin, µIU/mL	2.48 ^a	3.65 ^b	3.09 ^{ab}	0.25	< 0.01
IGF-I, ng/mL	112.9 ^a	143.6 ^b	137.3 ^b	7.3	0.03
Progesterone,					
ng/mL	6.38 ^a	7.79 ^b	7.75 ^b	0.36	0.01

¹ Within rows, values with different superscripts differ ($P \le 0.10$).



Figure 1. Plasma concentrations of urea N (PUN) in pregnant beef heifers consuming a low-quality cool-season forage (meadow foxtail) and receiving no supplementation (**CON**; n = 4) or supplements based on protein (**PROT**; n = 4) or energy ingredients (**ENER**; n = 4). A treatment × hour interaction was detected (P < 0.01) for PUN. Within hour, letters indicate the following treatment differences; a = PROT vs. CON (P < 0.01), b = ENER vs. CON (P < 0.02).

0.62). In addition, mean PUN concentration was greater (P < 0.01) for ENER and PROT heifers compared with CON, and similar (P = 0.44) between ENER and PROT (Table 3). Concentration of PUN is positively associated with intake of CP, RDP, and concentration of ruminal ammonia (Broderick and Clayton, 1997). Therefore, treatment effects detected for PUN can be attributed to the equivalent treatment effects detected for CP and RDP intake (Table 2).

Mean plasma glucose concentration was greater (P =0.03) for ENER and PROT compared with CON heifers, and similar (P = 0.96) between ENER and PROT (Table 4). Glucose concentration in beef cattle was positively associated with feed intake and rate of BW gain, as observed herein based on the greater nutrient intake and ADG of PROT and ENER compared with CON heifers (Table 2). However, starch is the major dietary precursor for glucose in ruminants; hence, it would be expected that ENER heifers had greater plasma glucose concentrations compared to PROT. Nevertheless, Huntington (1997) indicated that growing cattle are highly capable of synthesizing glucose from amino acids, such as those provided in the PROT treatment or produced by rumen microbes. Mean plasma insulin and IGF-I concentrations were greater ($P \le 0.08$) for PROT and ENER compared with CON heifers, and did not differ (P > 0.15) between PROT and ENER heifers (Table 3).

A treatment effect was also detected (P = 0.01) for plasma P_4 concentration. Progesterone concentrations on d 0 were significant covariates (P < 0.01) but did not differ (P = 0.98) among treatments (6.84, 6.84, and 6.99 ng/mL for CON, ENER, and PROT, respectively; SEM = 0.71), indicating that heifers from all treatment groups had similar plasma P₄ concentration prior to the beginning of treatment administration. Within samples collected on d 13, 15, 17, and 19, mean plasma P_4 concentrations were greater ($P \le 0.01$) for PROT and ENER compared with CON heifers, and did not differ (P = 0.93) between PROT and ENER heifers (Table 3). The main hypothesis of this experiment was that beef heifers consuming a low-quality cool-season forage and receiving a supplement containing an energy ingredient would have greater plasma P₄ compared with unsupplemented or cohorts receiving a supplement based on a protein ingredient. This hypothesis was developed based on the premise that energy ingredients such as corn favor circulating concentrations of glucose, insulin, and IGF-I (Molento et al., 2002), whereas insulin and IGF-I have been positively associated with circulating P₄ concentration. More specifically, IGF-I is known to stimulate luteal P₄ synthesis (Spicer and Echternkamp, 1995). Insulin also stimulates luteal P₄ synthesis (Spicer and Echternkamp, 1995), and alleviates hepatic P₄ catabolism by CYP2C and CYP3A enzymes (Cooke et al., 2012). In the present experiment, the lack of differences in plasma P₄ concentrations between ENER and PROT heifers, which were greater compared with CON heifers, can be directly attributed to the equivalent treatment effects detected for insulin and IGF-I. Hence, the ENER and PROT treatments utilized herein equally increased plasma P_{4} concentrations in pregnant beef heifers consuming a lowquality cool-season forage.

IMPLICATIONS

Beef cattle consuming low-quality cool-season forages had similar ruminal forage degradability and intake, performance, and physiological status if offered supplements based on soybean meal or corn at 0.5 % of BW.

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Performance of pregnant beef cows limit-fed diets containing wheat straw treated with two rates of anhydrous ammonia and wet distiller's grain¹

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ABSTRACT: The objective of this experiment was to evaluate the inclusion of wheat straw previously treated with 0.0%, 1.5% or 3.0% (wt/wt; DM basis) anhydrous ammonia in combination with wet distiller's grain in the diets of pregnant beef cows on performance and BCS during an 84 d feeding period. Pregnant (2nd trimester), Spring calving, Angus-cross cows (n = 132; age = 4.78 ± 2.36 yr; initial BW = 590 ± 54.1 kg; initial BCS = 6.0 ± 0.44), were stratified by age, BW, and BCS and assigned randomly to one of three dietary treatments. Treatments consisted of wheat straw-based diets limit-fed at 1.9% BW (DM basis): 1) 64.1% wheat straw (CON); 2) 64.1% wheat straw treated with 1.5% (wt/wt) anhydrous ammonia (1.5A); 3) 64.1% wheat straw treated with 3.0% (wt/wt) anhydrous ammonia (3.0A). Cow BW, BCS, 13th rib backfat thickness and rump fat thickness were evaluated on d 0 and 84 of the experiment. All cows were fed a common diet prior to initiation of the experiment and for 12 d after to account for differences in gut fill. Diets containing ammoniated wheat straw resulted in greater ($P \le 0.05$) total BW gain, ADG, and tended (P = 0.09) to result in greater BCS than CON. Total BW gain was greater ($P \le 0.05$) for cows fed 1.5A (72.4 kg) and 3.0A (73.8 kg) than CON (59.0 kg). Overall, ADG was greatest for 3.0A, intermediate for 1.5A and least for CON (0.77, 0.75, 0.62 kg/d respectively). Total BW gain and ADG responded linearly to anhydrous ammonia application (P < 0.01). However, the relative change in BW gain and ADG was greater from CON to 1.5A than from 1.5A to 3.0A thus indicating a diminishing response to anhydrous ammonia application (Quadratic, P = 0.10). Cow BCS tended (P = 0.09) to be greater in cows fed 1.5A and 3.0A, final 13th rib backfat thickness, and final rump fat thickness were not influenced ($P \ge 0.20$) by diet treatments. Under the conditions of this experiment performance of pregnant beef cows may be improved by applying anhydrous ammonia to low quality forages, such as wheat straw, at a rate as low as 1.5% (wt/wt) of the DM content.

Key words: anhydrous ammonia, cow performance, wheat straw

INTRODUCTION

The persistence of drought conditions across the southern Great Plains has renewed interest among beef cattle producers in chemical methods of enhancing the feeding value of lowquality forages. The application of anhydrous ammonia is one of the most common chemical methods utilized due to the availability of anhydrous ammonia and ability to treat large quantities of hay in a single application. Traditionally, application of anhydrous ammonia at a rate of 3.0% (wt/wt) on a dry matter basis has been recommended (Kuhl and Blasi, 1998: Lalman et al., 2004). However, proportionally greater improvements in forage quality have been reported at lower rates of anhydrous ammonia application by Paterson et al. (1981), Laytimi et al., (1984), Brown et al., (1987) and more recently in a large scale case study conducted by Waggoner et al. (2014). Evaluating the use of lower anhydrous ammonia application rates may be warranted as the cost of anhydrous ammonia has increased from an average of \$10.82/45.4 kg from 1985-1995 to \$29.65/45.4 kg from 2003-2013 (USDA, 2013). Additionally, there has been speculation among beef producers that the performance responses associated with feeding forages treated with anhydrous ammonia will be diminished in diets containing wet distiller's grain. Therefore the objective of this study was to evaluate the dietary inclusion of wheat straw previously treated with 1.5% or 3.0% (wt/wt) anhydrous ammonia in combination with wet distiller's grain on performance and body condition score of pregnant beef cows.

MATERIALS AND METHODS

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

Animals and Experimental Design

Pregnant (2^{nd} trimester), Spring calving, Angus-cross cows (n = 132; age = 4.78 ± 2.36 yr; initial BW = 590 ± 54.1 kg; initial BCS = 6.0 ± 0.44), were stratified by age, BW, and BCS and assigned randomly to one of three dietary treatments (4 pen replicates per treatment; 11 cows per pen). Treatments consisted of 3 wheat straw-based (**Table 1**) diets: 1) 64.1% wheat straw (**CON**); 2) 64.1% wheat straw treated with 1.5% (wt/wt) anhydrous ammonia (**1.5A**); 3) 64.1% wheat straw treated with 3.0% (wt/wt) anhydrous ammonia (**3.0A**). Diets were limit-fed (1.9% initial BW) once daily at 0800 h for

¹Appreciation is expressed to Elanco Animal Health,

Greenfield, IN for the generous donation of Rumensin 90 that was used in this research.

Table 1. Ingredient and nutrient composition of diets containing wheat straw treated with 0.0% (Control), 1.5% or 3.0% anhydrous ammonia (wt/wt; DM basis)

	1	Freatment	1
Item	CON	1.5A	3.0A
Ingredient composition, % DM			
Ground, wheat straw-CON	64.1	-	-
Ground, Wheat straw-1.5%	-	64.1	-
Ground, Wheat straw-3.0%	-	-	64.1
Wet distillers grain	19.9	19.9	19.9
Rolled sorghum	14.9	14.9	14.9
Calcium Carbonate	0.5	0.5	0.5
Salt	0.2	0.2	0.2
Vitamin and mineral premix ²	0.4	0.4	0.4
Nutrient composition, DM basis ³			
CP, %	11.8	14.9	16.2
NDF, %	44.51	42.59	42.05
ADF, %	34.20	35.25	35.24
Calcium, %	0.99	0.94	0.90
Phosphorus, %	0.30	0.29	0.30
Sulfur, %	0.29	0.27	0.31

¹Treatments consisted of 3 diets, limit-fed at 1.9% initial BW (DM basis) 1) CON = 64.1% wheat straw diet (no anhydrous ammonia): 2) 1.5A = diet containing 64.1% wheat straw previously treated with 1.5% (wt/wt) anhydrous ammonia: 3) 3.0A = diet containing 64.1% wheat straw previously treated with 3.0% (wt/wt) anhydrous ammonia.

²To account for differences in gut fill cows were fed a common diet prior to collection of initial weight and were fed a common diet for 12 d prior to collection of final weight. Thus, calculation of ADG is based on 96 days on feed.

²Premix supplied 150 mg head⁻¹ d⁻¹ Rumensin®

3Nutrient analysis conducted by SDK Labs, Hutchinson, KS

the duration (84 d) of the experiment and were formulated to exceed the nutrient requirements of gestating beef cows NRC (2000). Cows were maintained in 1033 m^2 earth floor pens with 0.65 m linear bunk space allocated per animal.

Anhydrous Ammonia Application

Round bales (n = 140) of wheat straw were placed in 2 stacks, in a 3, 2 arrangement. Three 9.1 m, 1.3 cm diameter braided-polyvinyl anhydrous hoses connected to a 1.9 cm. black iron cross adapted to fit an anhydrous ACME fitting (Fairbank Equipment, Wichita, KS), were secured at approximately equal distances along the length of the stack to facilitate application of anhydrous ammonia. The stacks were then covered with 6 mil black plastic and sealed with approximately 30 cm of soil along the bottom edge of the stack. Anhydrous ammonia was applied at the predetermined rates of 1.5 and 3.0% of DM (wt/wt) basis (1 stack of each). Stacks treated with anhydrous ammonia remained covered with plastic for 14 days. All wheat straw was then ground through a 5.1 cm screen using a commercial hay grinder (Haybuster, Jamestown, ND).

Data Collection

Cow BW was measured on d 0 and 84 of the experiment. All cows were fed a common diet prior to the initiation of the experiment and for 12 d after to account for any potential differences in gut fill. Therefore cows were weighed again on d 96 of the experimental timeline. All performance calculations were conducted using BW obtained on d 0 and 96 of the experiment. Cows were weighed at approximately 0900 h on each respective weigh date, prior to ration delivery. Body condition scores were assigned by two independent, qualified observers using a 9-point scale (1= extremely emaciated, 9 = extremely obese; Wagner et al., 1988) on d 0 and 84. In an effort to further quantify body condition status. Backfat thickness at the 13th rib and rump fat thickness at the midpoint between the tuber coxae (hip bone) of the ilium and the tuber ischia (pin bone) of the ischium were measured ultrasonically on d 0 and 84 using an Aloka 500V (Aloka Co., Ltd., Wllingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-12mm window). Ultrasound images were collected with Cattle Performance Enhancement Company (CPEC, Oakley, KS) software. Backfat and rump fat thickness were estimated with procedures that incorporated image analysis software (Brethour, 1994) that are an integral component of the CPEC software.

Diet samples were collected weekly and frozen at -20°C. Samples were composited at the conclusion of the experiment and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, ADF, Ca, P, and S.

Statistical Analysis

Performance and ultrasound data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Pen was utilized as the experimental unit. Polynomial contrasts were used to evaluate linear and quadratic effects of anhydrous ammonia application. Least square means are presented and differences were considered significant at $P \le$ 0.05. Tendencies were discussed when $0.5 < P \le 0.10$.

RESULTS AND DISCUSSION

Pregnant cows fed diets containing wheat straw previously treated with anhydrous ammonia resulted in greater ($P \le 0.05$) total BW gain and ADG, and tended (P = 0.09) to result in greater BCS than CON over the 84 d feeding period (Table 2). Total BW gain was greater ($P \leq$ 0.05) for cows fed 1.5A (72.4 kg) or 3.0A (73.8 kg) than for cows fed CON (59.0 kg). Overall ADG was greatest for 3.0A, intermediate for 1.5A and least for CON (0.77, 0.75, 0.62 kg/d respectively). The observed response in both BW gain and ADG were linear (P < 0.01). Total BW gain and ADG of 1.5A were not different from 3.0A (P > 0.05). The relative change in BW gain and ADG was greater from CON to 1.5A than from 1.5A to 3.0A thus indicating a diminishing response to anhydrous ammonia application (Quadratic; P =0.10). However, final BW and BCS change were not different among treatments (P > 0.05).

-		Treatment ¹		_		Significan	ce of contrasts ²
Item	CON	1.5A	3.0A	SEM	P-value	Linear	Quadratic
Number of pens	4	4	4				
Body weight ²							
Initial, kg	575.1	563.3	566.0	7.56	0.51	0.27	0.73
Final, kg	634.1	635.7	639.8	8.92	0.89	0.89	0.65
Total Gain, kg	59.0 ^a	72.4 ^b	73.8 ^b	3.97	0.01	0.01	0.10
ADG, kg/d	0.62 ^a	0.75 ^b	0.77 ^b	0.04	0.01	0.01	0.10
BCS ³							
Initial	5.86	5.87	5.92	0.07	0.78	0.90	0.49
Final	5.86 ^a	5.94 ^a	6.08 ^b	0.07	0.09	0.43	0.04
Change	0.00	0.08	0.16	0.04	0.13	0.30	0.08

Table 2. Performance of pregnant beef cows fed diets containing wheat straw treated with 0.0% (Control), 1.5% or 3.0% anhydrous ammonia on (wt/wt; DM basis) for 84 days

¹Treatments consisted of 3 diets, limit-fed at 1.9% initial BW (DM basis) 1) CON = 64.1% wheat straw diet (no anhydrous ammonia): 2) 1.5A = diet containing 64.1% wheat straw previously treated with 1.5% (wt/wt) anhydrous ammonia: 3) 3.0A = diet containing 64.1% wheat straw previously treated with 3.0% (wt/wt) anhydrous ammonia.

 2 To account for differences in gut fill cows were fed a common diet prior to collection of initial weight and were fed a common diet for 12 d prior to collection of final weight. Thus, calculation of ADG is based on 96 days on feed.

³Significance of polynomial contrasts for anhydrous ammonia application Linear = linear effect of anhydrous ammonia application, Quadratic = quadratic effect of anhydrous ammonia application

⁴BCS scale : 1 to 9 (1=emaciated, 9=obese; Wagner et al., 1988)

^{a,b}Within a row, means without a common superscript differ ($P \le 0.05$)

The response to treating low quality forage with anhydrous ammonia has been extensively evaluated and is characterized by an increase in forage CP concentration, greater DM digestibility, and an increase in DM intake (Lalman et al., 2004). In the present study, all cows were limit-fed at 1.9% of BW (DM basis) and thus DM intake was not a factor of interest. The greater BW gain and ADG of cows fed 1.5A or 3.0A most likely occurred as a result of greater digestibility of the wheat straw in the 1.5A and 3.0A diets. Waggoner et al. (2014) reported 35% and 49% greater IVDMD of wheat straw following application of 1.5% and 3.0% (wt/wt) anhydrous ammonia, respectively, in large scale case study conducted across 6 locations. Paterson et al. (1981) and Brown et al. (1987) both evaluated anhydrous ammonia application rates of 0.0%, 2.0%, 3.0% and 4.0% (wt/wt) and observed improved digestibility of forages treated with anhydrous ammonia application rates below 3% (wt/wt). Paterson et al. (1981) reported 28% and 24% greater DM digestibility of corn stalks treated with 2.0% and 3.0% anhydrous ammonia, respectively, compared to untreated cornstalks fed to lambs. Brown et al. (1981) reported an 8.3% and 9.1% greater in-vitro organic matter disappearance of grass hay following application of 2.0% and 3.0% (wt/wt) anhydrous ammonia, respectively.

Although final BCS tended (P = 0.09) to be greater in cows fed 1.5A and 3.0A, final 13th rib backfat thickness, and final rump fat thickness (Table 3) were not influenced ($P \ge 0.20$) by anhydrous ammonia application A tendency (P= 0.10) for a main effect was observed for 13th rib backfat thickness change. The observed change in 13th rib backfat thickness during the 84 d trial was greatest for 3.0A, intermediate for CON, and least for 1.5A (2.97, 2.17, and 1.91 mm, respectively).

IMPLICATIONS

The results of this study indicate that the performance of pregnant beef cows may be improved by applying anhydrous ammonia to low quality forages, such as wheat straw, at a rate as low as 1.5% (wt/wt) of the DM content. Additionally, these improvements in cow performance were observed in diets containing wet distiller's grain. Treating wheat straw with 3.0% anhydrous ammonia resulted in the greatest BW gain and ADG. However, the observed improvement in these variables in response to the 1.5% application rate suggests that application of 1.5% anhydrous ammonia (wt/wt; DM basis) may be optimal when anhydrous ammonia prices are relatively high.

Table 3.Effects of feeding diets containing wheat straw treated with 0.0% (Control), 1.5% or 3.0% anhydrous ammonia (wt/wt; DM basis) to pregnant beef cows for 84 d on 13th rib backfat thickness and rump fat thickness

_	Treatment ¹			_		Significance of contrasts ²	
Item	CON	1.5A	3.0A	SEM	P-value	Linear	Quadratic
Backfat thickness, mm							
Initial	5.55	5.64	5.77	0.39	0.92	0.87	0.71
Final	7.72	7.55	8.74	0.51	0.20	0.81	0.07
Change	2.17 ^{ab}	1.91 ^a	2.97 ^b	0.36	0.10	0.60	0.03
Rump fat, mm							
Initial	8.95	8.88	9.24	0.68	0.92	0.94	0.69
Final	24.75	24.92	26.15	1.37	0.73	0.92	0.43
Change	15.80	16.05	16.92	0.92	0.66	0.85	0.38

¹Treatments consisted of 3 diets, limit-fed at 1.9% initial BW (DM basis) 1) CON = 64.1% wheat straw diet (no anhydrous ammonia): 2) 1.5A = diet containing 64.1% wheat straw previously treated with 1.5% (wt/wt) anhydrous ammonia: 3) 3.0A = diet containing 64.1% wheat straw previously treated with 3.0% (wt/wt) anhydrous ammonia

²Significance of polynomial contrasts for anhydrous ammonia application Linear = linear effect of anhydrous ammonia application, Quadratic = quadratic effect of anhydrous ammonia application

^{a,b}Within a row, means without a common superscript tended to differ ($P \le 0.10$).

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Evaluation of novel nasal and intestinal inoculants for feedlot receiving calves¹

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INTRODUCTION

ABSTRACT:¹ The study evaluated the effects of novel nasal and intestinal inoculants on performance and health of feedlot receiving calves. Crossbred calves (n = 659, initial BW = 189 ± 0.63 kg) were blocked by 6 truckloads (104 to 114 calves per truckload) arriving on different days at the Clayton Livestock Research Center. All calves received metaphylactic antibiotic treatment at initial processing, and were assigned to 48 pens and 4 treatments in a randomized complete block design. Treatments (2 \times 2 factorial arrangement) were no inoculant (-PROB) or a novel feed-grade inoculant (+PROB), and no nasal inoculant (-NASAL) or a novel nasal inoculant (+NASAL). Both the nasal inoculant and the feed-grade probiotic were bacterial mixtures of Enterococcus faecium (EF510), Lactobacillus acidophilus (LA210), and Pediococcus acidilacticii (PA910) to supply 2×10^7 cfu per bacterium for each calf daily. Pens of calves assigned to +NASAL received a 5-mL spray of a gelatinous solution containing the bacterial mixture in each nasal cavity upon initial processing, and pens of calves assigned to +PROB received a diet top-dressed with the bacterial mixture from d 0 to 56. Pen of calves were weighed on d 0, 28, and 56, and morbidity was recorded throughout the experiment. Performance data was analyzed using linear mixed models, and morbidity was analyzed as categorical proportions using generalized linear mixed models. No NASAL × PROB interactions ($P \ge 0.15$) occurred for calf BW, ADG from d 28 to 56 and from d 0 to 56, DMI, and G:F. A tendency for a NASAL \times PROB interaction (P = 0.09) occurred for ADG from d 0 to 28. Calf BW, ADG, DMI, and G:F were not different ($P \ge 0.19$) between treatment main effects. For morbidity, a tendency for a NASAL × PROB interaction (P = 0.10) occurred for percentage of first medical treatment of calves, and a NASAL \times PROB interaction (P = 0.04) occurred for percentage of total medical treatment of calves. In summary, the novel nasal and feed-grade inoculants did not improve performance and health of feedlot receiving calves. Limited response to these inoculants could be due to insufficient doses of the bacterial mixture, and(or) due to metaphylactic antibiotic treatment and low incidences of morbidity (10%) and mortality (0.45%).

Key words: Cattle, Feedlot, Nasal inoculant, Probiotic

Demand for increased food products has pressured livestock production systems to improve efficiency. This pressure is accompanied by demands for safe and high quality products. Use of antibiotic feed additives is common practice to optimize production efficiency in the feedlot cattle industry. However, the Food and Drug Administration is implementing a voluntary plan for the livestock industry to phase out certain antibiotics (FDA, 2013). As consumer demand for organic and naturally raised products increases, it is anticipated that antibiotic use for improved production efficiency will decrease and be limited to therapeutic treatment. Therefore, use of alternative natural products to replace antibiotic feed additives for improved production efficiency and health warrants investigation. Alternative products of interest include probiotics or direct-fed microbials.

Non-pathogenic microorganisms may serve various functional roles in the gastrointestinal tract of animals, including nutrient digestion, pathogen exclusion, short-chain fatty acid production, compound detoxification, vitamin supplementation, and immune development (Savage, 1986; Stevens and Hume, 1998; Hooper et al., 2003; Flint and Garner, 2009). Krehbiel et al. (2001) reported that lactic acidproducing bacteria did not affect feedlot calf performance, but calves receiving lactic acid-producing bacteria during their first medical treatment were less likely to receive a second medical treatment. Also, previous research (Swinney-Foyd et al., 1999; Galyean et al., 2000) demonstrated that daily supplementation of diets with lactate producing and(or) lactate utilizing bacteria improved feed efficiency and ADG of feedlot cattle.

We hypothesized that health and performance would be improved when newly received feedlot calves are inoculated with lactate-producing bacteria that were previously identified to be present in the nasal cavity of healthy cattle. Therefore, the objective of this study was to evaluate the effect of a combination of novel nasal and intestinal inoculants on health and performance of newly received calves during the first 56 d in the feedlot.

MATERIALS AND METHODS

Animals and Facilities

Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee.

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The experiment was conducted at the Clayton Livestock Research Center (Clayton, NM) and used 659 crossbred calves from south-eastern Texas. A total of 6 truckloads of calves were received; the first 2 loads were steers and intact bulls, and loads 3 to 6 consisted of heifers. All calves were housed in 48 soil-surfaced pens (12 m \times 35 m) with 11 m of bunk space. Upon arrival, calves were given access to long stem wheat hay, 0.9 kg of a feedlot receiving diet (Table 1), and water until initial processing the following morning. At initial processing, all animals were individually weighed (Silencer, Moly Mfg. Inc., Lorraine, KS), uniquely identified with a tag in the left ear, identified with the designated pen tag in the right ear, vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea, bovine respiratory syncytial virus, parainfluenza-3 virus, viral diseases (Inforce 3 and Bovi-Shield GOLD 5; Pfizer Animal Health, Exton, PA), and dosed with the internal and external parasiticide, dormamectin (Dectomax; Pfizer Animal Health). Bull calves were castrated and horns were tipped if necessary. All calves received an anabolic growth implant (100 mg progesterone and 10 mg estradiol benzonate; Synovex C; Pfizer Animal Health) and received ceftiofur crystalline free acid (Excede; Pfizer Animal Health) subcutaneously in the base of the ear. The average BW (and SE) on the day of initial processing across all loads was 189 ± 0.63 kg.

Experimental Design and Treatments

The experiment was a randomized complete block design with a total of 48 pens (experimental unit) and 13 to 15 calves per pen (73 to 85 cm bunk space per calf). The blocking factor was 6 truckloads (104 to 114 calves per truckload) of calves that arrived on different days at the Clayton Livestock Research Center. Within each block, calves were randomly assigned to 8 pens, and pens of calves were randomly assigned to 4 treatments in a 2×2 factorial arrangement (12 replicated pens per treatment for all blocks). Treatments were no probiotic (-PROB) or a novel feed-grade probiotic (+PROB) in a factorial arrangement with no nasal inoculant (-NASAL) or a novel nasal inoculant (+NASAL). Both the nasal inoculant and the feed-grade probiotic were a mixture of the lactate-producing bacteria, Enterococcus faecium (EF510), Lactobacillus acidophilis (LA210), and *Pediococcus acidilacticii* (PA910), and supplied 2×10^7 cfu per bacterium for each calf daily. The bacteria were identified in healthy cattle after nasal swab samples were taken from more than 50 cattle at different feedlots in the High Plains, US. Calves assigned to +NASAL received a 5-mL spray of a gelatinous solution containing the bacterial mixture in each nasal cavity during initial processing. For pens of calves assigned to +PROB, a solution containing the bacterial mixture was applied (using a watering can) to the diet immediately after delivery to the feed bunk, and a shovel was used to mix the probiotic solution with the diet.

All calves were fed a feedlot receiving diet for the first 28 d and a feedlot grower diet from d 28 to 56 (Table 1). Daily estimates of the quantity of unconsumed feed remaining in the feed bunks were made for each pen at approximately 06:30, 12:45, and 18:30 daily. Bunks were managed to allow

Table 1. Composition of diets (DM basis)¹

Item	RECEIVING	GROWER
Ingredient, %		
Wet corn gluten feed ²	43.31	34.08
Corn grain, cracked	17.36	33.69
Corn distiller's grains	17.64	17.78
Corn stover	14.71	11.89
Limestone	2.32	1.98
Urea	0.34	0.52
Salt	0.27	0.26
Availa-4 ³	0.13	0.07
Beefmax-510 ⁴	0.05	0.05
Medicated supplement ⁵	0.05	0.03
Vitamin E	0.04	0.01
Vitamin AD	0.0004	0.0004
Nutrient ⁶		
CP, %	21.4	19.2
ADF, %	16.8	14.4
Ca, %	0.99	0.87
P, %	0.68	0.57
ME ⁷ , Mcal/kg	2.67	2.71
NE _m ⁷ , Mcal/kg	1.75	1.79
NE ⁷ , Mcal/kg	1.13	1.16

RECEIVING = feedlot receiving ration fed from d 0 to 28; GROWER = feedlot grower ration fed from d 28 to 56.

²Sweet Bran (Cargill Inc., Minneapolis, MN).

³Availa 4 (Zinpro Corp., Eden Prairie, MN) contained 51,500 ppm Zn, 28,600 ppm Mn, 18,000 ppm Cu, and 1,800 ppm Co.

⁴Beefmax 510 (Cargill Inc.) contained 1.8% Cu, 9% Zn, and 360 ppm Se.

⁵RECEIVING = decoquinate (Deccox; Zoetis Animal Health); GROWER = lasalocid (Bovatec; Zoetis Animal Health).

⁶Analyzed by Servi-Tech Laboratories (Amarillo, TX).

⁷ME, Mcal/kg = 0.01×(81.81-0.48×%ADF)×4.409×0.82;

NE_m, Mcal/kg = 1.37×ME-0.138×ME²+0.0105×ME³-1.12;

 NE_{g} , $Mcal/kg = 1.42 \times ME-0.174 \times ME^{2}+0.0122 \times ME^{3}-1.65$ (NRC, 2000).

trace amounts of feed in the afternoon (18:30) and no feed in the morning before the first feeding. The diet was mixed in an overhead mixer (Butler Oswalt Model 1830, Garden City, KS) and delivered to the pens twice daily with a feed truck fitted with 6 individual bins, each with an auger for dispensing feed. The first feeding started at approximately 07:00 and the second feeding at approximately 13:00. Samples of the complete diet were obtained for DM analysis (100°C for 24 h). Also, composited samples were analyzed for nutrients by a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX) every second week. Occasionally there were feed refusals at 06:30 before the morning feeding; any feed refusals at this time were collected, weighed and analyzed for DM to correct for DMI.

Management and Collections

On d 28 and 56, calves were individually weighed in a single animal squeeze chute (Silencer, Moly Mfg. Inc.) and each pen of calves was weighed simultaneously using a platform scale. Calf health was assessed daily by trained personnel using the DART system (Pharmacia & Upjohn Animal Health, Kalamazoo, MI) with some modifications. Criteria for the DART system is depression, anorexia, respiratory, and temperature. Criteria used to indicate further evaluation ("pull") for clinical disease were depression, abnormal appetite, and respiratory signs. Signs of depression included arched back, glazed eyes, difficulty moving, and a hanging head. Signs of abnormal appetite included lack of fill, failure to come to the bunk when feed was delivered, and weight loss. Respiratory signs included lethargic breathing, noisy breathing, and extended head and neck as described by Step et al. (2008). Calves that needed further evaluation were removed ("pulled") from their pen and taken to the handling facilities where they were weighed (Silencer single animal squeeze chute), their rectal temperatures recorded, and given severity a score of 0 to 3 for depression and respiration. Calves were warranted medical treatment when the severity score was 2 or greater for either depression or respiration, had a rectal temperature of 40.5°C or higher, and if BW gain was zero or negative relative to previous measurements. Calves were treated according to the Clayton Livestock Research Center's standard protocol or as directed by the attending veterinarian. On the first medical treatment, animals were given a combination of antibiotic (florfenicol) and a fast-acting non-steroidal anti-inflammatory (flunixin meglumine) in a single dose (Resflor Gold; Merck Animal Health, Summit, NJ). For the second medical treatment, animals were given a broadspectrum fluoroquinolone antimicrobial (enrofloxacin; Baytril 100; Bayer Animal Health Division, Shawnee Mission, KS). If a third medical treatment was warranted, the animal was permanently removed from the study and moved to a hospital pen for further medical treatment.

Statistical Analysis

Calf BW, performance, and DMI were analyzed statistically as a randomized complete block design using linear mixed models (SAS Inst. Inc., Cary, NC), and morbidity responses were analyzed as categorical proportions using generalized linear mixed models (SAS Inst. Inc., Cary, NC). Pen was the experimental unit. For all data, the statistical model included the effects of NASAL, PROB, and NASAL × PROB interaction; block was random. Differences were considered significant when P < 0.05. Mortality data could not be analyzed statistically because only 3 calves died resulting in inadequate variation in the data.

RESULTS

No NASAL × PROB interactions ($P \ge 0.15$) occurred for calf BW, ADG (from d 28 to 56 and from d 0 to 56), DMI, and G:F (Table 2). A tendency for a NASAL × PROB interaction (P = 0.09) occurred for ADG from d 0 to 28 (Table 2); calf ADG tended to be lower for +NASAL than –NASAL when receiving no intestinal inoculant (–PROB), and calf ADG tended to be lower for +PROB than –PROB when receiving no nasal inoculant (–NASAL). Calves receiving both inoculants (+NASAL and +PROB) had ADG that were not different to those of calves receiving no inoculants (-NASAL and –PROB).

For morbidity, a tendency for a NASAL \times PROB interaction (P = 0.10) occurred for percentage of first medical

treatment of calves, and a NASAL × PROB interaction (P = 0.04) occurred for percentage of total medical treatment of calves (Table 2). Percentage of calves removed for medical treatment were greater for +NASAL than –NASAL when receiving no intestinal inoculant (–PROB), and calves removed for medical treatment were greater for +PROB than –PROB when receiving no nasal inoculant (–NASAL). Morbidity of calves receiving both inoculants (+NASAL and +PROB) was not different to morbidity of calves receiving no inoculants (-NASAL and –PROB). Mortality was not analyzed statistically; 2 calves receiving -NASAL and +PROB died, and 1 calf receiving no inoculants died.

DISCUSSION

Morbidity, ADG, DMI, and G:F of calves receiving +NASAL and +PROB were not different to that of control calves, which indicated that the combination of novel nasal and intestinal inoculants evaluated in this study did not improve health and performance of newly received calves during the first 56 d in the feedlot. These results are consistent with those of Krehbiel et al. (2001), who observed no differences in health and performance of calves receiving 5×10^9 cfu lactic acid-producing bacteria (*Enterococcus faecium*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Bifidobacterium longum*) over a 42 d experimental period.

In the current study, all calves received metaphylactic antibiotic treatment upon initial processing. Antibiotic use for nontherapeutic application are generally administered during times of high disease risk such as upon arrival to a feedlot. Antibiotic treatment alters the microbial population, fermentation and metabolism within the rumen and might be the causative factor for the lack in response to inoculants. Furthermore, no response to nasal and intestinal inoculants could be due to an insufficient dose of the lactate-producing bacteria via the novel nasal and intestinal inoculant. Vasconcelos et al. (2007) evaluated effects of increasing the dose of live cultures of Lactobacillus acidophilus (NP 51) combined with a single dose Propionibacterium freudenreichii (NP 24) on performance of finishing beef steers. They found that G:F was improved with the Lactobacillus acidophilus (NP 51) at a dose of 1×10^7 cfu and 1×10^9 cfu, and that a dose of 1×10^8 cfu did not significantly affect G:F.

A tendency for lower ADG in calves receiving +NASAL than –NASAL when not receiving intestinal inoculant, and lower ADG in calves receiving +PROB than –PROB when not receiving nasal inoculant, appears to be due to greater morbidity in these calves. Lower ADG was consistent with a numerically lower DMI associated with morbid calves. According to Galyean and Hubbert (1995), calves that do not succumb to respiratory disease gain more rapidly than morbid calves, presumably as a result of greater DMI by healthy calves.

Results demonstrated that the nasal and feed-grade inoculants used in this study did not improve performance and health of receiving calves under well-managed feedlot conditions where calves received metaphylactic antibiotic treatment. Therefore, use of the nasal and novel feedgrade inoculants might be more effective in the absence of

Table 2. The effects of nasal and intestinal inoculants on performance and health of feedlot receiving cattle

	Treatments ¹			_		P-value		
	-NA	SAL	+NA	SAL				NASAL
Item	-PROB	+PROB	-PROB	+PROB	SEM	NASAL	PROB	× PROB
Pens	12	12	12	12				
BW, kg								
d 0	189.6	189.4	190.7	188.4	1.43	0.94	0.28	0.36
d 28	208.0	205.2	207.0	206.6	1.87	0.87	0.21	0.37
d 56	249.3	247.3	246.9	247.4	2.17	0.55	0.68	0.52
ADG, kg/d								
d 0 to 28	0.66	0.57	0.58	0.65	0.08	0.93	0.77	0.09
d 28 to 56	1.47	1.50	1.42	1.46	0.08	0.31	0.52	0.95
d 0 to 56	1.07	1.03	1.00	1.05	0.03	0.47	0.78	0.18
DMI, kg/d								
d 0 to 28	4.04	3.96	3.91	4.02	0.07	0.58	0.83	0.17
d 28 to 56	6.70	6.55	6.40	6.49	0.13	0.19	0.81	0.34
d 0 to 56	5.37	5.25	5.15	5.26	0.09	0.23	0.90	0.20
G:F								
d 0 to 28	0.161	0.143	0.148	0.159	0.018	0.91	0.68	0.15
d 28 to 56	0.219	0.229	0.221	0.225	0.010	0.85	0.24	0.55
d 0 to 56	0.198	0.197	0.194	0.200	0.004	0.91	0.56	0.31
Morbidity ² , %								
First treatment	7.01	13.01	9.86	8.14	3.27	0.78	0.38	0.10
Total treatment	7.52	14.70	10.94	8.06	3.62	0.61	0.43	0.04

¹Treatments were a 2 × 2 factorial arrangement of a novel nasal inoculant not given (-NASAL) or given (+NASAL) to calves during initial processing on d 0, and a novel feed-grade intestinal inoculant not added (-PROB) or added (+PROB) to the diets during the 56-d study.

²First treatment = percentage of first medical treatment of calves; Total treatment = percentage of total medical treatment (first and second combined) of calves. Mortality was not analyzed statistically because only 3 calves died resulting in inadequate variation in the data.

metaphylactic treatment or in the presence of "high-risk" calves with a greater percentage of morbidity and mortality.

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Effect of wheat middling supplementation on metabolizable protein of heifers grazing winter wheat pasture

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ABSTRACT: Eight runnially and duodenally cannulated, Angus-crossbred heifers $(272 \pm 19.2 \text{ kg})$ grazing winter wheat pasture (WWP) were used in a completely randomized design to evaluate effects of wheat middling (WM; offered at 0.4% of BW; as-fed basis) supplementation on metabolizable protein. The experiment was conducted from April 5 through April 19, 2013. Heifers grazed in a single WWP with supplements offered individually, once daily at 0700 h. Forage DM, total OM, NDF intake, and OM intake expressed as g/kg of BW were not affected ($P \ge 0.11$) by WM supplementation, and total CP intake (P = 0.03) increased with WM supplementation. Supplementation of WM increased the flow of OM, and CP $(P \le 0.04)$ to the small intestine, but not the flow of NDF (P = 0.40). Feed protein flowing to the small intestine was not affected (P = 0.26) and microbial protein tended to increase (P = 0.10) with WM supplementation. Total tract OM, CP, and NDF digestibility were not affected ($P \ge 0.13$) by WM supplementation. Ruminal pH decreased and total VFA production increased (P = 0.01) with WM supplementation. Ruminal ammonia (P = 0.65), molar proportion of VFA (P \geq 0.11), and acetate to propionate ratio were not affected (*P* = 0.25) by WM supplementation. Supplementation of WM to cattle grazing WWP improves metabolizable protein by increasing microbial protein synthesis.

Key words: metabolizable protein, wheat middling, winter wheat pasture

INTRODUCTION

Grazing winter wheat (*Triticum aestivum*) in the southern Great Plains of United States has become a common practice and has great agricultural and economical importance as 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), available carbohydrates are rapidly fermented in the rumen, and it contains 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 readily digestible carbohydrate content, it is considered insufficient in energy due to the low structural carbohydrate content (Shroyer et al., 1993).

Wheat middling (**WM**) is the byproduct of wheat milling industry and it is high in readily digestible fiber and low in starch (Amaral-Philips and Hemken, 1997). Therefore, we hypothesized that WM supplementation to cattle grazing WWP would result in greater microbial protein synthesis by compensating the energy deficiency of WWP thus improving metabolizable protein. Therefore, the objective of this experiment was to evaluate effects of WM supplementation on metabolizable protein of heifers grazing WWP.

MATERIAL AND METHODS

All procedures and experimental protocols were approved by the New Mexico State University Institutional Animal Care and Use Committee. Eight Angus cross heifers (272 \pm 19.1 kg) fitted with ruminal and duodenal cannulas were used in a complete randomized design. Heifers were assigned randomly to 1 of 2 treatments: 1) Control or not supplemented, and 2) supplemented with wheat middling at 0.4% of BW. The experiment consisted of one 15-d experimental period; the first 11 d were used for adaptation to wheat pasture grazing and supplement, and the last 4 d for sample collection. The experiment was conducted from April 5 through April 19, 2013. Heifers grazed a single wheat pasture (Triticum *aestivum*), with supplement offered individually once daily at 0900 h. Each day, heifers were gathered into a holding pen, tied to the holding pen fence with 1-m long halter, and fed individually their allotted supplement. Supplement was offered in a feed tub. Heifers were allowed access to their supplement for 30 min, after which uneaten supplement was placed into their rumens through the ruminal cannula.

Two heifers were placed in a holding pen for ruminal evacuations at 0900 h 1 d before beginning the experimental period. Digesta was placed in plastic bags lining 133-L plastic containers. After evacuation, heifers returned to pasture and were allowed to graze for 60 min. Masticate samples were subsequently collected. Masticate samples were dried in a forced-air oven (50°C) to a constant weight, ground in a Wiley mill (2-mm screen, Wiley mill model 4, Thomas Scientific, Swedesboro, NJ), and composited on an equal dry-weight basis.

Gelatin capsules containing chromic oxide (8 g) were dosed ruminally, twice daily (at 0700 and 1900) on d 6 to 15 of the experiment periods. Chromic oxide was used to estimate nutrient digestibility. Ruminal fluid samples were collected at 0 (before dosing), 3, 6, 9, 12, 15, and 21 h after dosing. Ruminal fluid pH was determined immediately after collection, and the samples were then acidified with 7.2 N NH₂SO₄ at a rate of 1 mL/100 mL of ruminal fluid and cooled (4 °C) in whirl pack bags for later analysis of ammonia, and VFA.

Duodenal and fecal samples were collected during the collection period with 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; and d 15, 0400 h. Individual samples consisted of approximately 100 mL of duodenal chyme and 200 g (wet basis) of fecal material. Each type of sample from each heifer was composited for 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). Ruminal fluid/saline solution mixture was cooled (4 °C) for later bacterial isolation.

Laboratory Analysis

Duodenal, fecal and bacterial samples were thawed, mixed, subsampled, and dried in a freeze dryer (-50°C) for 96 h, and ground in a Wiley mill (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). Also, samples were 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). Duodenal samples were analyzed for purines (Zinn and Owens, 1986). Isolated ruminal bacteria were analyzed for DM, N, ash, and purines as previously described. Ruminal fluid samples were centrifuged at 27,000 × g for 20 min and analyzed for NH₂-N (Broderick and Kang, 1980), and VFA (Goetsch and Galyean, 1983). Ruminal fluid/ saline mixture was centrifuged at $1,000 \times g$ for 10 min to remove feed particle and protozoa from the supernatant. Supernatant was centrifuged at $27,000 \times g$ for 20 min to separate bacteria from the supernatant, and then the isolated bacteria was resuspended in saline and centrifuged at 27,000 \times g for 20 min. The final isolated bacteria were collected and freeze-dried (-50°C) for 96 h.

Calculations

Daily fecal DM output and chyme DM leaving the abomasum were calculated using fecal and duodenal Cr concentration, respectively. Fecal DM output was calculated by dividing Cr dose by fecal Cr concentration. Similarly, chyme DM leaving the abomasum was calculated 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 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. Methane production (mol/ mol glucose equivalent fermented) was estimated based on the theoretical fermentation balance for observed molar distribution of VFA (Wolin, 1960).

Statistical Analysis

Data were analyzed as a completely randomized design with GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effect of wheat middling. Effects were considered significant when $P \le 0.05$.

RESULTS AND DISCUSSION

Effect of WM supplementation on characteristics of ruminal and total tract digestion are shown in Table 1. Forage DM, total OM, NDF intake, and OM intake expressed as g/kg of BW were not affected ($P \ge 0.11$) by WM supplementation. These results are consistent with previous results (Chabot et al., 2005), which reported that supplementing readily digestible fiber sources to cattle grazing high quality grass such as winter wheat did not affect intake. Conversely, total CP intake increased with WM supplementation ($P \le 0.03$). The reason for the greater CP intake was that WM had greater CP concentration than forage.

Flow to the duodenum was increased for OM and CP (P ≤ 0.04) but not for NDF flow ($P \geq 0.40$). When partitioning the CP flowing to the small intestine, feed CP (313, and $388 \pm$ 42.5 g/d for control and WM supplementation, respectively) was not affected (P = 0.26), and microbial protein (762, and 864 ± 37.8 g/d for control and WM supplementation, respectively) tended to increase (P = 0.10) with WM supplementation. Even though wheat pasture is high in soluble carbohydrate and CP, but it is considered insufficient in energy because of its low structural carbohydrate contents (Johnson et al 1973; Reuter and Horn 2000). Microbial protein synthesis is principally affected by ruminal concentration of N-containing compounds and the quantity of OM available for fermentation (Burroughs et al., 1974). Therefore, we hypothesized that supplementing energy using WM as readily digestible fiber source to cattle grazing WWP would provide energy to optimal microbial protein synthesis. Total tract OM, CP and NDF digestibility were not affected (P >0.13) by WM supplementation.

Ruminal pH (6.5, and 6.2 ± 0.03 for control and WM, respectively) decreased and total VFA production (94.4,

	WM ¹ , %	6 of BW		
Item	0	0.4%	SE	P-Value
DM intake, g/d				
Forage	10958	10629	344.5	0.52
WM	-	1010	-	-
Total	10959	11729	341.9	0.16
OM intake, g/d				
Forage	9468	9183	297	0.52
WM	-	1058	-	-
Total	9468	10242	295	0.11
Total OM intake g/kg of BW	33.9	37.3	1.43	0.15
CP intake, g/d				
Forage	1369	1328	43	0.52
WM	-	207.2		-
Total	1369	1535	42.5	0.03
NDF intake, g/d				
Forage	6552	6355	206	0.52
WM	-	378.7	-	-
Total	6552	6734	204.9	0.55
Flow to the duodenum, g/d				
DM	4003	4775	160	0.01
OM	2897	3474	112	0.01
NDF	958	841	95	0.41
CP	1076	1252	45	0.03
Microbial protein	762.3	863.7	37.8	0.10
Feed protein	313.3	388.1	42.5	0.26
Microbial protein efficiency ²	18.6	20.6	1.2	0.29
Ruminal digestion, %				
OM	55.9	51.9	1.85	0.18
CP	76.9	74.6	3.16	0.61
NDF	85.5	87.4	1.34	0.34
Fecal excretion, g/d				
OM	1687	1827	53.9	0.11
CP	338	373	6.5	0.02
NDF	1153	1261	50.7	0.18
Total tract digestion, %				
OM	82.1	82.1	0.14	0.94
CP	75.2	75.6	0.33	0.45
NDF	82.4	81.3	0.44	0.13

Table 1. Effect of wheat middling supplementation on characteristic of ruminal and total tract digestion of beef heifers grazing winter wheat pasture

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 $\frac{\text{NDF}}{^{1}\text{WM}} = \text{wheat middling supplementation: } 0 = \text{no supplementation; and } 0.4 = \text{WM offered } 0.4\% \text{ BW.}$ $^{2}\text{ Microbial protein efficiency} = \text{Duodenal microbial N, g.kg-}^{1}\text{ OM fermented in the rumen.}$

and 111.2 \pm 3.17 m*M* for control and WM, respectively) increased (*P* = 0.01) with WM supplementation. The lower pH and greater VFA production might suggest more rapid degradation rate for WM supplementation (Fieser and Vanzant 2004). While ammonia production (*P* = 0.65), molar proportion of VFA (*P* \geq 0.11) and acetate to propionate ratio were not affected by WM supplementation (*P* \geq 0.25).

In summary, WM supplementation improved metabolizable protein of cattle grazing WWP by improving microbial protein.

IMPLICATIONS

Results from this experiment imply that WM supplementation as a source of readily digestible fiber increases metabolizable protein of cattle grazing WWP, and therefore it is expected to improve productive performance.

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Predicting the forage intake by lactating beef cows¹

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ABSTRACT: The current NRC model to estimate the DMI by cows is based on a single equation related to metabolic size and net energy density in the diet. However, research has indicated that the observed DMI by grazing cows can be influenced by animal demands, largely determined by BW, physiological state, genetics, or any combination of the 3. The lactating cow has the highest nutrient requirements of all the physiological states she can experience. A broad-based database was developed from published experiments where forage intake was reported for lactating cows. New equations were developed using stepwise regression modeling. It was found that cow BW only explains a portion of the variation noted in forage intake; regression modeling showed that herbage CP, calf weaning weight, calf ADG, and milk production significantly ($P \le 0.11$) affected forage intake. Forage intake models that included cow milk production explained the most variation ($r^2 = 0.32$; 3% downward bias). However, milk production is difficult to measure in field applications. Hence, a model was constructed using calf weaning weight as a segregate for milk production. The model using calf weaning weight accounted for less variation $(r^2 = 0.28)$ than the milk production model, but only had a 1% downward bias. Hence, the new NRC models to predict the forage intake by lactating beef cows will need to contain other independent variables than just cow BW; models probably will need to contain independent variables that quantify the metabolic demands that are inherent to the cow's physiological state.

Key words: beef, cows, forage intake, pastures, rangelands

INTRODUCTION

Predicting forage intake by grazing cattle is important to the enterprise, but difficult because of the impossibility of directly measuring intake (Coleman et al., 1999, 2014). Prediction quality of forage intake by cattle grazing native rangelands has become a prominent issue in recent years, not only because it is useful to predict cattle performance and supplementation requirements (NRC, 1996), but because knowing forage intake allows producers to utilize rangelands without overgrazing. Residual herbage mass on rangeland not only protects the rangeland from wind erosion, but provides escape cover to threatened wildlife species and improves water infiltration, and sustains the following years' forage production potential (Adiku et al., 2010; Thacker et al, 2012; Wine et al., 2012).

In a review by Coleman et al. (1999), they noted that intake varies not only due to feed quality and physical characteristics, but also due to the animal's physiological state. Further, Coleman et al. (2014) constructed forage intake models including daily milk production or calf weaning weight and they showed considerable promise compared to models based solely on cow BW. Therefore, any attempt to predict intake probably must take in both considerations, cow size and physiological state, and at best provide an estimate of the forage intake under the specified conditions. The NRC committees included a model for intake in the NRC (1984) and (1996) publications and due to limitations, later modified the model (NRC, 2000). Major limitations of the model included the fact that all the experimental data that were included were from pen-fed animals, many on high-concentrate diets.

Observational field data and peer reviewed research have identified deficiencies in the 1996/2000 Nutrient Requirements of Beef Cattle (NRC, 1996, 2000). Lardy et al. (2004) reported that predicted energy deficits for summercalving cows grazing either range or pasture in November and were biologically unreasonable. In most instances, field data of cow performance agree with the findings of Lardy et al. (2004), though there has been some research reported where predicted forage intake for dormant forage exceeded estimated forage intake indirectly measured with external markers (Bodine and Purvis, 2003).

The following research is to evaluate the possibility of using independent variables that indicate nutrient demand in forage intake models for lactating beef cows. Further, quality of predictions was evaluated by regressing observed values against predicted values.

¹Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

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MATERIALS AND METHODS

A database was compiled from 32 experiments containing a total of 264 group means, conducted both on pasture and in confinement, with all or nearly all forage diets. The only assumption made was that when forage intake was explicitly characterized as DM, then it was transformed to OM intake by multiplying forage DMI by 0.92. These data are summarized in Table 1. Some of the animals from pasture research received supplemental feed. In those instances where cattle were supplemented, dietary digestibility and CP concentration was assumed to be the same as that reported for forage samples; hence, no adjustment in diet nutritive value was made for supplement, but forage intake was recorded as the total forage and supplement. While this procedure ignored potential associative effects (positive for increased DIP and negative for starch), the major objective of this project was to determine the feasibility of using independent variables of cow performance from grazing/conserved forage research that indicate nutrient demands in forage intake models compare to the existing model, not to develop a final model to be used in the new NRC for publication. Moreover, methodology is often confounded with location and pasture conditions of the experiment. The confounding of location and pasture condition was not evaluated for this paper, but it would need to be considered before incorporating into a more enduring predictive model (Coleman et al., 2014). To achieve convergence, any experiment with only one treatment group was eliminated (n = 3) and some of the records did not have all the necessary data, especially forage digestibility and CP, used to construct the model.

The remaining data (n = 194) were used for an overall analysis in Proc Reg (SAS Int., Inc.; Cary, NC) with the selection option set equal to stepwise. In the stepwise process, independent variables were recruited when $P \le 0.30$ and were excluded from the model when $P \ge 0.15$. The first model constructed included cow BW and calf ADG to re-evaluate a model suggest by Coleman et al. (2014). The second model constructed included cow BW, forage CP, and forage digestibility to evaluate the effects forage quality effect on forage intake. The third model constructed included cow BW, cow ADG, and average daily milk production to evaluate the effects of maternal nutrient demands on forage intake. Last, the forth model constructed included cow BW, calf weaning weight, and calf ADG to evaluate the effects of maternal nutrient demands on forage intake when milk production data is unavailable. For models 3 and 4, simple regression analysis was used as a measure of association between the observed forage intakes and the predicted forage intakes. Further, model bias was measured by the β_1 coefficient of a simple regression with a zero intercept resulting from the regression of observed values on predictions as described in Gunter and Galyean (2000).

RESULTS AND DISCUSSION

The first model constructed included cow BW and calf weaning weight to re-evaluate the model suggest by Coleman et al. (2014). This model used 194 of the observations and had a $r^2 = 0.23$. The model is as follows:

Forage intake (kg of OM) = 4.41 + 0.00942CowBW + 3.57CalfADG (1)

Where CowBW is initial cow BW (kg) and CalfADG is calf pre-weaning ADG (kg); both independent variables were included in the model and were highly significant (P < 0.01). The model produced by this database dose not account for as much variation as the model produced by Coleman et al. (2014). In the later research, the authors' model explained 64% of the variation and was a better predictor than milk production [forage OM intake (kg/d) = 1.9 + 12.8CalfADG (kg)]. Further, cow BW was not a significant impendent variable in their model.

The second model constructed included cow BW, forage CP, and forage digestibility to evaluate the effects forage quality effect on forage intake. This model used 155 of the observations and had a $r^2 = 0.03$. The model is as follows:

Forage intake (kg of OM) = 10.78 + 0.00674CowBW + 0.0511ForageCP (2)

Where CowBW is initial cow BW and ForageCP is the forage CP (% of OM); only the independent variable, ForageCP, was included in the model and was modestly significant (P = 0.11). When only these 2 independent variables were made available to the model, forage digestibility was not included because it never met the significance ($P \le 0.30$) criteria. Diet digestibility has been shown to be moderately related to DMI because diets with lower digestibilities usually contain more

Table 1. S	Summary of v	variable com	piled in the	database before	any truncation	for model	construction
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Variable	N	Mean	SD	Minimum	Maximum
Cow BW, kg	264	485	95.3	294	735
Cow ADG, kg	185	0.23	0.528	-1.31	2.13
Milk production, kg/d	197	6.8	2.95	1.2	21.2
Forage CP, % of OM	157	12.3	4.65	4.8	23.4
Forage digestibility, % of OM	245	61.8	8.78	30.7	78.0
Calf weaning weight, kg	192	209	42.8	134	317
Calf ADG, kg	194	0.81	0.248	0.39	1.67
Forage OM intake, kg/d	264	11.9	3.65	3.7	25.1

cell walls (Van Soest, 1982). This premise is stronger with diets that are less than 60% digestible. With forages diets, cells wall concentration, NDF, and its digestibility is has been shown to be inversely correlated with OM digestibility (Van Soest, 1982). However, the diets in our database range from 31 to 78% digestible. Diets with a digestibility greater than 60% have been shown to have physiological controls regulating intake (Conrad et al., 1966). Hence, over a wide range of diet qualities, forage digestibility is not a strong determinate of forage intake for lactating beef cows.

The third model constructed included cow BW, cow ADG, and average daily milk production to evaluate the effects of maternal nutrient demands on forage intake. This model used 138 of the observations and had a $r^2 = 0.34$. The model is as follows:

Forage intake (kg of OM) = 3.51 + 0.0131CowBW + 0.314Milk (3)

Where CowBW is initial cow BW (kg) and Milk is average milk production (kg/d); both independent variables included in the model were highly significant (P < 0.01). Based on the comparison with predicted values regressed on observed values, this model accounted for 27% of the variation noted in forage intake. Further, the regression analysis also showed a 3% downward bias with predicted values produced by the model.

Cow performance can be measured indirectly by calf production, particularly weaning weight, but the direct output from the cow to the calf is milk (Coleman et al., 2014). Milk production was a positive driver for cow OM intake and accounted for 56% of the variation or more in adjusted intake in other research (Wagner, 1985; Coleman et al., 2014). Milk production is difficult to measure even though it is listed in most registration EPD. However, the calculation for EPD are not based on milk production, but the residual after growth potential of both sire and dam were accounted for with best linear unbiased prediction models. Therefore, it makes little sense to try to include milk in a general prediction equation for an important economic expense like feed intake. A surrogate could be calf weaning weight, but in these data, calf ADG is more closely related to milk production. So, models predicting forage intake using a segregate may be useful.

The last model constructed included cow BW, calf weaning weight, and calf ADG to evaluate the effects of maternal nutrient demands on forage intake when milk production data is unavailable. This model used 184 of the observations and had a $r^2 = 0.29$. The model is as follows:

Forage intake (kg of OM) = 2.31 + 0.00483CowBW + 0.0346CalfWW (4)

Where CowBW is initial cow BW (kg) and CalfWW is calf weaning weight (kg); both independent variables included in the model were significant, but CalfWW was more so (P < 0.01) than CowBW (P = 0.11). Calf ADG was not included in the model because they never met the significance ($P \le 0.30$) criteria. Based on the comparison with predicted values regressed on observed values, this

model accounted for 27% of the variation noted in forage intake. Further, the regression analysis also showed only a 1% downward bias with predicted values produced by the model.

As stated in latest issue of Nutrient Requirements of Beef Cattle (NRC, 2000), further research is needed to develop more accurate equations to predict forage intake by cattle eating forage diets. A considerable amount of research has been completed since 2000; however, much remains to be done, especially with lactating cows. It is quite apparent from this analysis and other research (Coleman et al., 1999, 2014) that forage intake by grazing beef cows is not static and not determined simply by BW. Forage intake varies with physiological state, level of milk production, and to some extent forage quality. Significant progress was made by the NRC committee in developing intake prediction equations in the past 2 publications (NRC, 1996, 2000). The next model developed for grazing beef cattle will be an important improvement over the current model, but it will also have shortcomings given the plethora of biological types of cattle grazing the extensive variety of environments throughout the world; I believe the most significant limitation to the NRC modelers will be fundamental and descriptive data over a wide range of environments needed to produce high-quality predictions.

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Cobalt supplementation in pre-weaned beef calves affects humoral immune response and feedlot health¹

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ABSTRACT: Economic losses from morbidity and mortality associated with bovine respiratory disease (BRD) in beef cattle are approaching \$2 billion annually. Mineral supplementation during pre-weaning has potential to reduce sickness and improve health. The mineral cobalt (Co) is used by rumen-inhabiting microbes for the production of vitamin B_{12} . Vitamin B_{12} is a cofactor for vital metabolic pathways in tissue carbohydrate and lipid metabolisms required for maintenance and growth. Vitamin B₁₂ is also vital for B cell proliferation to form plasma cells that secrete antibodies. Recent studies have shown increases to NRC-recommended Co supplementation levels enhanced antibody response in weaned beef calves. The objective of this study was to evaluate if an orally-supplied Co (30 g) bolus dosed preweaning affects the humoral immune system during the postweaning feeding period and reduces the incidence of BRD. Five different ranches with similar genetics, forage, mineral, water aquifer bases, and the same preconditioning health program were utilized. Two hundred, six to eight month old beef calves were randomly selected from 2,000 head (BW 220 ± 24 kg). All calves were vaccinated for *Mannheimia* haemolytica three weeks before weaning. At vaccination one hundred calves were randomly selected to receive an oral Co (30 g) sustained release bolus and one hundred calves randomly selected as controls. Both treatment and control calves were bled at vaccination to analyze initial M. haemolytica leukotoxin antibody titers and again at d 70 in the feedlot. All calves were weaned and transported the same day. Calves were fed the same rations during the feeding period. There was 42% increase (P = 0.06) in M. haemolytica leukotoxin antibody titer in calves treated with Co. Feedlot health (morbidity and mortality) was evaluated pre-weaning and daily during the feeding period. Co treatment decreased (P = 0.02) the incidence of BRD.

Key words: bovine respiratory disease, humoral immune response, *Mannheimia haemolytica* leukotoxin

INTRODUCTION

In ruminants, rumen microbes utilize Co for the biosynthesis of cobalamin compounds. Cobalamins are

vitamers of vitamin B_{12} and are important to the metabolism of both microbes and their ruminant hosts (Underwood and Suttle, 2004). Vitamin B_{12} , which is absorbed in the ileum, is a vital cofactor in specific metabolic enzymes in lipid and carbohydrate energy metabolism (Underwood and Suttle, 2004). Vitamin B_{12} is also utilized for B cell proliferation to form plasma cells that secrete antibodies (Tamura, 1999; Fernandes, Jolly, and Lawerence, 2008). Cattle may naturally obtain cobalt from forages depending on the availability of Co in the soil (Van Soest, 1982).

Cobalt deficiency is strongly associated with geographic and geologic variations in the element's availability and concentration in the soil (Suttle, 2010). The bioavailability of Co can also be impacted by antagonistic interactions with other minerals (Suttle, 2010). Iron, manganese, zinc, and iodine in soil or water have been described as antagonists for Co absorption with antagonistic interactions in cattle further predisposing to Co deficiency (Puls, 1999). Numerous beef cattle production areas in the Unites States, including many parts of Florida and Montana, have insufficient Co levels or have too higher concentrations of antagonistic minerals (Kubota and Allaway, 1972). Present NRC recommendations are set at 0.1 ppm DM for beef cattle (Maas, 2005).

Interactions between the immune system and microminerals in beef cattle are extremely complex but necessary for normal immune response to infection and disease (Engle, 2001; Tiffany et al., 2003). Concentrations of micro minerals in beef cattle need to be maintained within narrow limits to preserve the functional integrity of cells and tissues vital to normal immune function (Underwood and Suttle, 2004; McDowell, 2008). Micro-mineral deficiencies in beef cattle have been associated with decreased immune system function (Engle, 2001; Blezinger, 2010). These deficiencies require the animal to metabolically compensate for the nutrient deviation, resulting in depressed immune function, and diminished animal health and performance (Engle, 2001). A deficiency impairs the ability of the immune system in normal phagocytic function, in innate immunity, and in cytokine production during adaptive immunity. In a previous study, we showed Co supplementation significantly affected humoral responses to a single antigen (P < 0.01) (Sager, 2013). Bovine respiratory disease (BRD) has

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a major financial impact on the cattle industry causing up to 70% of animal losses in small feedlots and increasing costs through treatment of associated morbidities (Galyean et al. 1999). BRD is exacerbated by the additive effects of weaning and transportation stresses that each negatively impact humoral immune responses and cortisol production (Galyean et al., 1999). These stress responses can persist for several weeks placing cattle at increased susceptibility for developing BRD (Mackenzie, et al. 1997). Therefore treatments that enhance humoral immune function may reduce BRD-related morbidity and mortality.

MATERIALS AND METHODS

Care, handling, and sampling of the animals were done in accordance with the Montana State University Animal Care Committee and utilizing BQA (Beef Quality Assurance) recommendations (MSU 05-2013). This study was conducted in natural range conditions on five ranches (Wilsall and Ringling, MT) and at the Weber Feedlot in Sanborn, MN. All animals that were randomly selected for this study survived through completion of the study. Study design was implemented to reduce as many variables as possible utilizing similar genetics, age, herd- health program, and same forage, aquifer, and mineral programs. All calves were immunized within a 10-d period, turned back on their mothers for 20 to 25 d, then weaned, and transported the same day to one feedlot in MN.

Animals

Five different ranches with similar genetics, forage, mineral, water aquifer bases, and the same pre-conditioning health program were utilized in this study. Genetically the calves were from ranches that used the same seed stock breeding of Angus-sired calves from Angus X Simmental cross cow herd. Genetics have been similar for over ten years to meet requirements for consignment to the same buyer each year. Two hundred, 6- to 8-mo-old beef calves were randomly selected from 2,000 head (BW 220± 24 kg). All calves were vaccinated for Mannheimia haemolytica (Vista Once, Merck Animal Health, Milsboro, DE) 3 wk before weaning. At vaccination (d 1, September 21, 2013) 100 calves were randomly selected to receive orally a Co (30 g) sustained release bolus (Animal Health Supplies, Airedale, Australia) and 100 calves randomly selected as controls. Both treatment and control calves were bled by coccygeal vein (2 mL) at vaccination to analyze initial M. haemolytica leukotoxin antibody. On October 15th (on d 25) all calves were weaned, loaded on a tractor trailer and transported the same day (transported by truck 1,666 km to Sanborn, MN). At d 70 feedlot calves were re-bled per coccygeal vein (2 mL) to evaluate *M. haemolytica* antibody response during Co supplementation. Blood was chilled in cold pack boxes to allow normal clotting and centrifuged at 24 h at 2,500 rpm for 20 min. Serum was collected and refrigerated until samples were mailed for M. haemolytica leukotoxin antibody analysis. Antibody response was measured by ELISA antileukotoxin antibody (M. haemolytica leukotoxin) described below (as anti-leukotoxin titers are preferred for evaluation of a *M. haemolytica* response) by Oklahoma State University Diagnostic Laboratory, Stillwater, OK). Calves were fed to grow approximately 1.4 to 1.8 kg/d (NRC recommendations) on a diet of corn silage, protein supplement, and ground corn.

Calves were from identical health programs (Vista Once, Merck Animal Health, Milsboro, DE and Vision 7 + Somugen, Merck Animal Health, Molsboro, DE) involving immunization at branding (April, 15-May, 1) and pre-weaning (September 15-October 1) of each yr.

Forage, water, and mineral programs were identical as calves were raised in the same geographical area (48 km radius, with same forage and aquifer base) and the cow herd fed the same mineral program during calf production.

Results were analyzed and compared to objectives of the study. Incidence of sickness and prevalence of sick days were measured in the calves as daily animal health was tracked and recorded during the trial. Sick calves were not excluded from the study as natural infection should provide a more robust antibody response to natural infection.

Leukotoxin (LKT) antibody assay

Antibody titers to M. haemolytica leukotoxin were determined by enzyme-linked immunosorbent assays (ELISA). The M. haemolytica A1 strain used for antigen preparation was originally isolated from a feedlot calf. Formalinized M. haemolytica was prepared from a washed 24-h culture by suspending cells in 0.4% formalinized saline at a concentration determined spectrophotometrically to be 1.850 OD₆₅₀. LKT was prepared from culture supernatant from a 3-h culture of M. haemolytica A1 grown in RPMI-1640 medium at 37° C in a shaking incubator. The LKT was partially purified by precipitation with 40 to 60% ammonium sulfate as previously described (Confer, 1997). The precipitate was resuspended in 3M guanidine containing 59 mM NaHPO₄ and 100 mM NaCl. By SDS-PAGE of the LKT preparation, one intensely staining band was identified at 105 kDa and confirmed to be LKT on a western blot using an anti-LKT monoclonal antibody (Confer et al., 1997). Leukotoxic activity was 10⁴ LKT Units per ml (Clinkenbeard et al., 1994). The 2-keto-3-deoxyoctonate concentration was 7.5 µg/mg of protein. Wells of 96-well microtiter plates were coated with whole cells at an optical density reading equivalent to 108 CFU of a 24-h culture or with LKT at 50 ng per well. Sera were diluted in PBS-Tween 20 containing 1% BSA and tested at dilutions of 1:800 for whole cells and 1:1600 for LKT. The extent of antibody binding was detected using a 1:400 dilution of horseradish peroxidaseconjugated, affinity purified rabbit anti-bovine IgG. Antibody responses are expressed mean $OD_{490} \pm SD$. Leukotoxin titers for *M. haemolytica* were completed by Oklahoma State University; Marie Montelongo, laboratory technician, under the direction of Anthony Confer PhD.

Statistical Analyses

M. haemolytica leukotoxin antibody data was analyzed by a Welch Two Sample t test with variances and differences considered significant at P < 0.05. Calf was the experimental unit. Response to Co treatment for proportions of calves that developed BRD was analyzed using contingency chi square.

RESULTS AND DISCUSSION

The current study used an orally-administered sustained Co bolus that has an average lifespan of 120 d, although individual differences in calf rumen microbiota and microbial digestion would affect life-time utilization of the sustained bolus. Co supplementation above NRC recommendations during pre-weaning resulted in increased *M. haemolytica* leukotoxin serum antibody levels and a significant reduction in BRD occurrence between treatment and control calves. We observed a 42% increase in *M. haemolytica* leukotoxin serum antibody levels) at d 70 in the treatment group (1.682 pg/mL serum compared to 1.168 pg/mL of serum) compared to the control group, and this trended toward significance (P = 0.06).

Animal health was tracked daily throughout the trial and the incidences of sickness and prevalence of sick days were recorded. Sick calves were not excluded from the study as natural infection should provide a more robust antibody response to natural infection with *M. haemolytica*. Consistent with improved *M. haemolytica* leukotoxin serum antibody levels, treatment calves had significantly decreased BRD diagnoses (P = 0.02) and improved health compared to control calves during the feeding period. Variations in *M. haemolytica* leukotoxin antibody were observed. The variation is believed to be due to a drop in leukotoxin antibody titer levels after d 60, however, this physiological response was not known until leukotoxin analysis was completed in January, 2014.

Improved antibody production in weaned beef calves fed supplemented Co above a NRC requirement is probably a direct result of increased vitamin B_{12} production by rumen microbial synthesis. Improved feedlot health and decreased sickness has previously been shown to positively impact carcass quality characteristics, such as Grade, Yield, and HCW (Stovall et al., 2000; McBeth et al., 2002, Smith, 2009). BRD costs the beef industry because of affected animals that increase cost of production, have reduced performance, and reduced carcass quality (Stovall et al., 2000). Because of differences found in the health status between treatment and control groups improved carcass characteristics would be expected in the treatment group.

Records on treatment duration, dosage, and product used were recorded during the study by Weber Feedlot management. During the feeding period there was above usual weather stress with below average temperatures and increased windy days during the first 45 d of the feeding period and could have increased BRD. However, we did not observe increased BRD in either group of calves.-However, foot rot infections were more common than usual in both groups and likely resulted from increased periods of ice and frozen ground conditions increasing foot abrasions and cuts allowing *Fusobacterium* spp to increase virulence and morbidity rates from normal years.

Additional studies using increased number of calves and sampling them within the 60 d post-vaccination period



Figure1. Cobalt control calves (mean 1.168 pg / ml) and cobalt treatment calves (with mean titer 1.667 pg / ml). Co treatment calves showed a 43% increase in mean Mannheimia haemolytica leukotoxin antibody titer. (P = 0.06).



Figure2. Chart showing number of BRD calves in control verses treatment groups.

would improve our understanding of the effects of Co supplementation in beef calves. Improved health should correlate to improved carcass characteristics at harvest, but this remains to be tested. Cobalt supplementation above NRC recommendations resulted in improved health during the feeding period. Our findings warrant further investigation and indicate that NRC-recommended Co levels may need to be increased for improved beef calf health and decreased BRD problems in newly arrived beef calves.

IMPLICATIONS

This study is relevant to the U.S. Beef Cattle Industry in that these results indicate that increased NRC Co may decrease incidence of BRD, improve health in weaned beef calves, improve feedlot health and growth, and improve carcass characteristics. Each of these impacts would increase the profitability in beef calf production. Previous NRC Co recommendations were derived from studies during the 1950's when beef cattle production goals were much different than today. Results from this study indicate that current NRC Co levels should be increased to improve post weaned health and performance in beef calves.

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Effects of monensin sodium and plant extracts containing cinnamaldehyde, eugenol and capsicum oleoresin on days to puberty, average daily gain and feed efficiency in developing beef heifers receiving a high roughage diet

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INTRODUCTION

ABSTRACT: The objectives of this study were to determine the influence of monensin sodium and a combination of the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol on feed efficiency, BW gain and age at puberty in developing beef heifers. One-hundred-and-seven Angus heifers, blocked by weight, were randomly assigned to one of four treatment groups. All heifers received the same high roughage diet. Treatments were applied as a top-dressed supplement to the roughage diet fed immediately after the base diet. There were 4 treatments: 1) monensin sodium (MON) 200 mg·hd⁻¹·d⁻¹, 2) plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (CCE) administered at 11 g of plant extracts daily, 3) control (CON) delivered as a non-medicated base mix, and 4) combination (COMB) of monensin sodium (200 mg) and natural plant extracts (11 g) ·hd⁻¹·d⁻¹. Heifers were weighed and serum samples collected every 11 d, except during the first period which was 8 d. Serum samples were collected from the same subset of heifers (n = 75) across the four treatments to analyze progesterone concentrations by RIA. Data were analyzed using the GLIMMIX procedure of SAS. Treatments were arranged as a 2 x 2 factorial in a randomized complete block design. There were no differences in initial BW (281.1 \pm 28.2 kg) or final BW (359.1 \pm 35.2 kg). Differences were observed between treatment groups in ADG from d 11 to d 22 and from d 44 to d 66 (P < 0.05), however, over the entire experiment there was no difference (P > 0.05) in ADG. Dry matter intake was different among treatments from d 0 to d 8. Overall gain to feed ratio was not different (P > 0.05). No differences were seen for age of puberty (P > 0.05) as determined by estrus detection patch status evaluated every 11 d. Results indicate no difference between administering monensin sodium or plant extracts to developing beef heifers. This indicates that plant extracts may be a suitable substitute for feeding ionophores. Previous research has clearly demonstrated improvements in ADG, feed to gain ratio and age at puberty in heifers fed monensin sodium, yet this effect was not observed in heifers fed monensin sodium in this study. Therefore, caution should be used in making conclusive changes in nutritional management or adoption of plant extracts in heifer development programs based solely on this study.

Key words: beef heifer development, feed efficiency, monensin sodium, plant extracts, puberty

Age of puberty is a major determinant of the lifetime productivity of a beef female (Schillo et al., 1992). Current beef management practices in the United States are for heifers to calve by two years of age (Funston and Deutscher, 2004). Feed resources account for one of the greatest costs of production while developing heifers (Clark et al., 2005). Heifers reaching a BW of 50 to 57% of their mature body weight, in comparison to the traditional target weight of 60 to 65% by the breeding season can reduce development costs without impairing reproductive performance (Funston and Deutscher, 2004). Increasing ADG and improving feed efficiency can aid in heifers reaching target breeding weight at a younger age. Ionophores, such as monensin sodium, have been used in beef diets because of their ability to increase feed efficiency and nutrient utilization (Bergen and Bates, 1984). When monensin sodium is fed feed conversion increases, propionate to acetate ratio increases, and protein degradation decreases (Raun, 1990). Monensin sodium has been shown to decrease the age of puberty (Lalman et al., 1993; Moseley et al., 1982; Purvis and Whittier, 1996) and increase first service conception rate (Purvis and Whittier, 1996). However, there is some controversy over feeding monensin sodium due to being classified as an antibiotic (Edrington et al., 2003) and some beef marketing programs do not allow feeding of antibiotics (Cattle Today Online, 2014). With this in mind, researchers have continued to extend studies and have discovered that plant extracts have some antimicrobial properties that may allow manipulation of microbial activity in the rumen, making them a potential alternative to ionophores (Benchaar et al., 2008).

Plant extracts, such as cinnamaldehyde, capsicum oleoresin and eugenol, have been studied both *in vivo* and *in vitro* (Cardozo et al, 2006; Yang et al, 2010; Yang et al, 2010b). Cinnamaldehdye is the main active component of cinnamon, capsicum oleoresin is derived from red peppers and eugenol is extracted from cloves. Geraci et al. (2012) found that ADG was increased among all treatments by 16% from d 45 to 84 when utilizing plant extracts (266 mg·hd⁻¹·d⁻¹ cinnamaldehyde and eugenol, plus 133 mg·hd⁻¹·d⁻¹ capsicum oleoresin) in a finishing ration. Dry matter intake and water intake were found to be significantly (P = 0.03) decreased when feeding cinnamaldehyde in combination with eugenol (Cardozo et al., 2006). Yang et al., (2010)

found that cinnamaldehyde helped reduce the ruminal crude protein (CP) degradation allowing for undegraded proteins to be absorbed in the small intestine. This process could be beneficial for growing animals.

Currently, the published literature regarding the performance of animals fed plant extracts is limited. There is little published on how plant extracts influence age of puberty in beef heifers. Our objectives in this study were to determine the influence of monensin sodium and a combination of the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol on feed efficiency, BW gain age at puberty in beef heifers.

MATERIALS AND METHODS

Animals and Diet

This project was approved by the Institutional Animal Care and Use Committee at Colorado State University. Onehundred-and-seven Angus heifers (n = 107), blocked by weight, were randomly assigned to one of four treatment groups using a random number generator. Pens were also randomly assigned a treatment group. There were 4 replicates per treatment. Once heifers were assigned their treatment group, they were then placed into their assigned pen. Pens had either six or seven heifers for 84 total days on feed.

All heifers received the same high roughage diet. Treatments were applied as feed supplements that were topdressed on the base ration to each pen immediately after the base ration was fed. All supplements were composed of a dried distillers grain base (Table 1). The four treatments were: 1) monensin sodium (MON) fed 200 mg hd⁻¹ d of monensin 2) natural plant extracts cinnamaldehyde, capsicum oleoresin and eugenol (CCE), commercially available as RumeNext® (ADM Alliance Nutrition Inc., Quincy, IL), fed to deliver 11g hd⁻¹ d of plant extracts, 3) control (CON) fed at 317.5 g·hd⁻¹ ¹·d⁻¹ as a base mix, and 4) combination (COMB) of monensin sodium (200 mg) and the natural plant extracts (11 g) fed at 317.5 g·hd⁻¹·d⁻¹. The ration was 13.0 % wheat straw, 12.6% alfalfa hay, 50.5% corn silage, 14.0% whole corn, 7.4% dried distillers grain, 0.10% limestone, and 2.4% liquid mineral supplement (Table 1). The ration had a 63.7% DM content. Feed refusals were recorded and feed offered was adjusted daily based on evaluation by a trained bunk reader to minimize feed refusals. Feed samples and ORTS were collected weekly and intake was calculated on an as fed by pen basis. The DM and nutrient analysis reported was performed by SDK (SDK Laboratories, Hutchinson, KS).

Data Collection

Heifers were weighed every 11 d to measure ADG and feed efficiency. Blood samples (10 mL) were collected every 11 d from the same subset of heifers (n=75) spread equally across treatment groups for later analysis of progesterone concentrations by radioimmunoassay (RIA). Blood was collected via jugular venipuncture and was promptly centrifuged at 2,500 x g for 25 min at room temperature. Serum was separated and frozen for later analysis that is not completed at this writing. Progesterone concentration \geq 1ng/

 Table 1. Ingredient composition and proximate analysis of the basal diet fed to developing beef heifers.

Item	
Ingredient ¹	%
Wheat straw	13.0
Alfalfa hay	12.57
Corn silage	50.5
Whole corn	14.0
Dried distillers grain	7.43
Minerals	2.5
Proximate analysis ²	
DM, %	67.97
CP, %	32.03
NE _G Mcal/lb.	0.44
NE _M Mcal/lb.	0.76
TDN, %	67.98
ADF, %	29.21
Supplement ³	
CP, %	26.00
Crude Fat, %	9.50
Crude Fiber, %	12.50
Calcium (min), %	2.25
Calcium (max), %	2.75
Phosphorous, %	0.75
Salt (min), %	5.00
Salt (max), %	6.00

¹Compositon of ration on an as fed basis.

²Analysis of ration on a dry matter basis.

³Treatments: Medicated premixes were added to the base ration to supply: MON = 200 mg·hd⁻¹·d⁻¹, CCE (RumeNext®, ADM Alliance Nutrition Inc., Quincy, IL) =cinnamaldehyde, capsicum oleoresin and eugenol at 11 g·hd⁻¹·d⁻¹, CON = no monensin or plant extracts, COMB = both monensin and plant extracts delivered at 200 mg·hd⁻¹·d⁻¹ and 11 g·hd⁻¹·d⁻¹, respectively. Supplement premixes also consisted of minerals such as potassium, magnesium, manganese, zinc, cobalt, copper, iodine, selenium, vitamin A and vitamin E, in very discrete amounts.

mL will be used to confirm puberty onset. Estrus detection patches (Estrotect[™] Heat Detector, Rockway Inc., Spring Valley, WI) were applied on day 0. Patch status was recorded every 11 d as activated, not activated or missing. If a patch was reported as missing during the 11 d weigh d, it was replaced with a new patch. Age at puberty was estimated based on Estrotect[™] patch status.

Empirical observations of cattle fed the plant extracts used in this study had suggested a calming effect on animal behavior. Therefore, observations were made in the current study to evaluate change in heifer behavior and chute exit characteristics objectively every 11 d using an infrared sensor timing system (FarmTek Inc., North Wylie, TX) that measured chute exit velocity to see if treatment supplement had an effect on docility. Time was measured in m/s. Velocity was calculated 1.83 m from the front of the head catch and ending 3.66 m beyond the head catch.

Data Analysis

Data were analyzed using the GLIMMIX procedure (v93; SAS Institute Inc., Cary, NC). The study was set up as a 2x2 factorial with a randomized complete block design. Heifers were blocked by initial BW. Treatment was a fixed

effect. Average daily gain, DMI, gain:feed and pubertal onset were the response variables. If P < 0.05, the treatment effects were considered significant. A trend was noted if P < 0.10.

RESULTS AND DISCUSSION

Feedlot Performance

There were no differences in initial BW (281.08 \pm 28.22 kg) or final BW (359.09 ± 35.2 kg) in this experiment. Slight differences were observed between treatment groups in ADG from d 11 to d 22 and from d 44 to d 66 (P < 0.05) (Table 2). From d 11 to 22, heifers in the CCE group had significantly decreased ADG compared to those in the CON group (P < 0.05) and there was a trend (P = 0.06) for MON heifers to have slightly higher ADG in comparison with CCE heifers. There was a tendency for the COMB (0.24 kg) and CON (0.49 kg) groups to have greater ADG in comparison with the CCE treatment group. The heifers receiving CCE demonstrated a decreased ADG from d 44 to 66 in contrast to the other treatments (P = 0.05). During this 2 wk period, MON tended to have greater ADG (1.22 kg). Consistent with this data, Moseley et al., (1982) noted that heifers fed monensin sodium expressed an increased ADG compared to a control group and heifers fed monensin sodium under restricted DMI. In contrast, Geraci et al., (2012) reported animals receiving plant extracts exhibited higher ADG compared to those receiving monensin sodium. Geraci et al., (2012) also noticed that ADG was only significant among treatments at the end of the study from d 45 to 84. This observation, along with the results from this study, lead to the assumption that there may be a time by treatment interaction when feeding monensin sodium, or plant extracts. Similarly, Yang et al., (2010b) reported that while there was no difference in ADG among treatments over the entire experiment (P = 0.08), it was quadratically changed during the first 28 days of the study and the high dose (1600 mg·hd⁻¹·d⁻¹) cinnamaldehyde (CIN) treatment group performed lower than the medium and low dose groups (800 and 400 mg·hd⁻¹·d⁻¹, respectively). It was concluded by the authors that the effects of CIN were dose dependent (Yang et al., 2010; Yang et al., 2010b). It is possible that animal response to plant extracts not only interacts with time, but also are dose dependent.

Dry matter intake did not show interaction (P > 0.05) and was only different among treatments from d 0 to d 8. Treatments groups that did not receive monensin sodium supplementation showed higher DMI (P = 0.05). Heifers fed CCE tended (P = 0.09) to have the highest DMI. During the second week of the trial, MON heifers showed a tendency (P = 0.06) for decreased DMI in contrast to those fed CON or CCE (1.92 kg). The COMB heifers showed the lowest DMI. From d 22 to the end of the trial, there were no differences among treatment groups when analyzing DMI. Similarly, authors in a separate study stated that there were fluctuations in DMI of heifers fed monensin sodium or plant extracts from week 1 to 4, but then DMI was not affected from day 29 to the end of the experiment (Yang et al., 2010b). In another study (Yang et al., 2010), the authors observed that the effects of CIN on DMI were dose dependent. In contrast, Cardozo et al. (2006) combined CIN plus eugenol as a treatment group, which decreased DMI as well as water intake (P < 0.03). The reductions in water intake could be associated with the reduction in DMI. It is possible that Cardozo et al. (2006) saw different results than the Yang et al. (2010 and 2010b) because of the combination of CIN and eugenol compared with feeding solely CIN (Yang et al., 2010). The CCE treatment group could have varying effects because it is a combination of three plant extracts; perhaps combining plant extracts causes their biological features to perform differently, or have a different mode of action in the ruminant. Cardozo et al. (2006) observed that CIN had the ability to increase total VFA concentration and decrease the acetate:proprionate ratio, as well as the NH₂-N concentration. This suggests that CIN may improve nutrient utilization in the rumen. It is possible that rumen microbes develop resistance over time to the plant extracts. The fluctuations in DMI could also be due to ulterior factors such as stress or weather.

The gain to feed ratio was not significantly different (P > 0.05). This is expected since the ADG and DMI were also not found to be significantly different. Although not significant, heifers fed monensin sodium in both the MON and COMB treatment groups had the greater feed efficiency compared to heifers in the CCE and CON treatment groups. This result could be due in part to the increased ADG and decreased DMI that MON heifers demonstrated during specific time periods throughout the study.

Chute exit velocity was measured to evaluate heifer's docility and escape inclination to assess whether or not treatment affected animal behavior or docility. Exit velocity differences among treatments groups were not different (P > 0.05). To date there is very little literature about the effects of monensin sodium and plant extracts on docility. More research would need to be conducted to know if these results were expected.

There was no difference observed in age at puberty due to supplement fed (Table 2). These results are not consistent with previous research. Several studies have reported a decrease in age at puberty in heifers fed monensin sodium. Lalman et al. (1993); McCartor et al. (1979); Moseley et al. (1982); Moseley et al. (1977); and Purvis and Whittier (1996) all reported decreased age and weight at puberty in heifers fed monensin sodium. One possible explanation for not observing a difference in age at puberty in the current study may be that as a result of genetic selection for younger age at puberty in beef heifers by selection pressure on bull scrotal circumference, puberty in heifers is now occurring at an age younger than the threshold that was previously overcome nutritionally by feeding an ionophore. This argument appears to have merit based on reported age of puberty of 377 d in 1982 (Moseley et al., 1982) and 369 d as reported by Lalman et al (1993) in heifers fed monensin sodium, while the age at puberty in heifers in the current study was 315 d, an approximate 60 d decrease. A second explanation for no observed difference in age of puberty in the current study may be that the method used to detect age at

	Treatment ¹				Р	-		
	MON	CCE	CON	COMB	SEM ²	Interaction	MON	CCE
DMI, kg								
d 0 - d 8	8.48	9.90	9.50	8.39	0.31	0.68	0.05	0.80
d 8 - d 15	8.36	9.87	10.36	8.03	0.48	0.93	0.06	0.66
d 15 - d 22	9.48	10.56	13.41	9.64	0.78	0.34	0.13	0.39
d 22 - 32	11.25	11.81	12.17	11.77	0.52	0.71	0.68	0.95
d 32- d 38	11.12	11.90	12.11	12.17	0.48	0.56	0.73	0.70
d 38 - d 43	11.53	11.42	12.40	11.63	0.34	0.47	0.66	0.55
d 43 - d 50	12.02	12.05	12.81	12.34	0.39	0.54	0.78	0.80
d 50 - d 57	12.27	12.09	13.07	12.66	0.39	0.43	0.89	0.73
d 57 - d 64	12.27	12.87	13.09	13.09	0.40	0.57	0.74	0.74
d 64 - d 71	11.81	12.68	12.82	12.32	0.43	0.73	0.47	0.85
d 71 - d 84	12.72	13.10	13.17	13.41	0.45	0.71	0.95	0.77
ADG, kg								
d 0 - d 11	1.76	1.98	2.00	1.85	0.05	0.75	0.35	0.92
d 11 - d 22	0.45	-0.04	0.49	0.24	0.06	0.36	0.47	0.05
d 22 - d 33	0.96	1.15	0.92	1.07	0.07	0.79	0.93	0.58
d 33 - d 44	0.70	0.59	0.53	0.88	0.09	0.85	0.56	0.69
d 44 - d 66 ³	1.22	0.79	1.05	0.87	0.06	0.78	0.39	0.05
d 66 - d 77	1.11	0.88	1.03	0.93	0.06	0.96	0.85	0.50
d 77 - d 84	0.19	0.71	0.21	0.44	0.10	0.73	0.72	0.39
G:F								
	0.10	0.09	0.08	0.10	0.002	0.68	0.08	0.77
Age of puberty, d	322.8	313.5	313.6	310.8	2.5	0.20	0.50	0.20
Exit velocity, m/s	0.82	0.89	0.86	0.80	0.02	0.66	0.43	0.76

 Table 2. The effects of monensin sodium and the plant extracts cinnamaldehyde, capsicum oleoresin and eugenol on gain to feed, exit velocity and age of puberty in yearling beef heifers

¹Treatments: MON = 200 mg·hd⁻¹·d⁻¹, CCE (RumeNext®, ADM Alliance Nutrition Inc., Quincy, IL) =cinnamaldehyde, capsicum oleoresin and eugenol at 11 g·hd⁻¹·d⁻¹, CON = no monensin or plant extracts, COMB = both monensin and plant extracts delivered at 200 mg·hd⁻¹·d⁻¹ and 11 g·hd⁻¹·d⁻¹, respectively

2Standard error of the mean.

³ADG was adjusted from d 44 to d 66, omitting d 55 data due to malfunctions with the chute scale.

⁴Exit velocity was measured 1.83 m from the head catch to 3.66 m from the head catch.

puberty (activated estrous detection patches monitored at 11 d intervals) was not adequate to identify differences between heifers fed monensin, plant extracts or a combination when compared with control heifers.

IMPLICATIONS

Results observed in this study indicate that there is no difference in weight performance or age at puberty between administering monensin sodium or plant extracts to developing beef heifers. This indicates that plant extracts may be a suitable substitute for feeding ionophores. Further research should be conducted on plant extract's mode of actions and pathways within the ruminant animal. Perhaps these results would answer how plant extracts can improve pubertal onset, and feed efficiency, in developing beef heifers in the future. This information would be valuable to animal scientists, as well as the producers who are always looking for ways to decrease their input costs in heifer development. Previous research has clearly demonstrated improvements in ADG, feed to gain ratio and age at puberty in heifers fed monensin sodium, yet this effect was not observed in heifers fed monensin sodium in this study. Therefore, caution should be used in making conclusive changes in nutritional management or adoption of plant extracts in heifer development programs based solely on this study.

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Performance of raising dairy calves by adding 15% ground alfalfa to calf starter

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ABSTRACT: The objective of this study was to evaluate the effects of 15% of ground alfalfa (Medicago sativa L.) mixed in calf starter ration on Holstein calf behavior and performance. Performance measures were calf birth weight (CBW), weight at 60 d (W60), ADG from birth to 60 d of age, and total weight gain (TWG). The experiment was conducted at the ICA-UABC Farm Facility, Baja California, Mexico. Sixty dairy calves (n = 26 females and n = 34 males) at two days of age were assigned randomly to each of two groups: group 1 (n = 31) fed with calf starter ad libitum, and group (n = 29) fed ad libitum with calf starter and alfalfa meal mixed 15 %. All calves were fed 2 L of colostrum at 6 and 12 h. Both groups of calves were given 2 L of whole milk every 12 h birth to weaning. Calf starter (14.78 %) and calf starter meal mixed (15.46%) was provided beginning at two days age. Calf birth weight was recorded as well as weight and weaning 60 d age. Statistical analyses were carried out by GLM procedure of the Statistical Analysis System program (SAS Institute, Inc.). Average weights at birth, weaning, total, and average daily gain $(40.8479 \pm 1.2972 \text{ and } 38.6940 \pm 1.2$ $1.3166 \text{ kg}; 63.2794 \pm 1.5496 \text{ and } 61.9742 \pm 1.5728; 22.4314$ \pm 1.1448 and 23.2801 \pm 1.1620 kg; and 60 d 0.3867 \pm 0.0277 and 0.4013 ± 0.0200 kg), respectively were not statistically different (P > 0.05) of treatments for either of the analyzed traits. These results based in limited numbers, suggest an economical evaluation. Also future research is suggested to determine the long term effects of diets containing forage.

Key words: dairy calves, ground alfalfa, calves starter, rearing calves.

INTRODUCTION

The intake of solid feed is vital to the calf for making the transition from a preruminant animal to a functioning ruminant. However, there is still much controversy concerning the composition of starter that should be fed to preruminant calves, especially regarding the level of forage those diets should contain (Montoro et al., 2013). Forage intake promotes muscular development of the rumen (Tamate et al., 1962; Hamada et al., 1976) and stimulates rumination and flow of saliva into the rumen (Hodgson, 1971). However, forage digestion by microorganisms does not provide sufficient concentrations of rumen VFA, especially butyrate, required for optimal papillae development. Fermentation of concentrates provides the necessary butyrate to stimulate papillae development, but these feeds may promote keratinization of the papillae in calves and lambs (Thompson et al., 1958; Nocek et al., 1980).

The amount of roughage necessary in the diet of young calves is unclear (Klein et al., 1987). Trials investigating the use of forage in starter rations have yielded inconsistent results. It is well documented (Kincaid, 1980; Thomas and Hinks, 1982; Stobo et al., 1985) that forage addition to the diet increases starter intake; however, other authors have reported a negative impact of forage addition on the intake of starter rations (Hibbs et al., 1956; Whitaker et al., 1957; Leibholz, 1975).

The particle size of ration influences the ruminal environment, volatile fatty acid production, and papillae structure and its function. Diets that are chopped or ground to fine particle sizes decrease the rumen pH and cellulolytic bacteria populations (Beharka et al., 1998). This decrease in pH, is caused by a lack of rumination and saliva flow into the rumen in both calves and mature cows (Kellaway et al., 1977; Santini et al., 1983). Ruminal papillae of animals receiving small forage particles have increased keratinization (McGavin and Morrill, 1976a). This decrease in active tissue results in a reduction in VFA absorption (Hinders and Owen, 1965; Nocek et al., 1980; Greenwood et al., 1997b). Papillae begin to branch to compensate for the loss in metabolically active tissue (Mc Gavin and Morrill, 1976b; Greenwood et al., 1997b; Beharka et al., 1998).

Determining the proper level of forage, to include in starter diets for optimal rumen development will benefit to the producer greatly by shortening the length of time that calf requires milk replacer, and also allow early weaning of the calf. There are several advantages of early weaning when compared to the prolonged feeding of milk replacer. Labor is greatly reduced when calves are fed starter rations when compared to feeding milk replacer. When calves are weaned earlier have fewer digestive problems (Klein et al., 1987). If optimal levels of forage are added to ration of raising dairy calves, it will provide the conditions to the proper development of the rumen, a better and mature digestive system of calves; it also will help to dairy producers to wean younger and health calves. This should promote the fed intake and growth after weaning. The objective of this study was to determine the effects of 15% of ground alfalfa mixed in calf starter ration of Holstein calf behavior and performance.

MATERIALS AND METHODS

The experiment was conducted at the ICA-UABC Farm Facility, Baja California, Mexico. Sixty dairy calves 60 Holstein calves were used (n = 26 females and n = 34 males). Calves were born in August 2011 to March 2012). Calves both females and males were assigned at random to treatments, beginning at two days of age: group 1 (n =13 females and n = 18 males) fed a calves starter ration (14.78 % CP ad-libitum, group 2 (n = 13 females and n = 16males) received a calves starter ration 15.46 % CP, it was added 15% ground alfalfa mixed ad libitum (Table 1). All calves after birth were separated from mothers and housed at individual pen in raising room, they were given 2 L of colostrum at 6 and 12 h old, were later provided 2 L of whole milk every 12 hours until weaning (60 d of age). We recorded the weight of calves at birth, and 60 d of age to estimate their weight, daily gain (ADG), and total weight gain (TWG. Data were analyzed as a completely random design using the GLM procedure of SAS v. 6 (SAS Inst. Inc. Cary, NY, 2002).

RESULTS AND DISCUSSION

Table 2 shows the mean and standard deviation of the results on the CBW (37.8461 ± 5.8288 and 39.6764 ± 7.3473 kg), at W60 (61.6923 ± 7.2375 and 63.8823 ± 7.9535 kg), ADG (0.4111 ± 0.1093 and 0.4173 ± 0.0955 kg) and TWG (23.8461 ± 6.3415 and 24.2058 ± 5.5420) of calves

females and males at this study, respectively. No significant differences (P>.05) were found between the two sexes. These results in agreement to other studies concerning to a strong effect on the sex of calves, since males are heavier 7 to 8% at birth than females calves.

The mean and standard deviation of the results on CBW and W60 in the animals under study (group 1 and 2), are presented in Table 3. The CBW in both groups were not different (P > 0.05), the results as averages were as follow: 40.8479 ± 1.2972 and 38.6940 ± 1.3166 kg in calves fed: the calves starter and calves starter mixed with 15% ground alfalfa, respectively. The W60 showed no significant difference (P >0.05) in the groups in, the average values were as follow: 63.2794 ± 1.5496 and 61.9742 ± 1.5728 kg in Group 1 and 2, respectively. Coverdale et al. (2004), found low averages at W60 d when fed initiator was added or in concentrate 7.5 and 15% bromegrass hay ground that the initiator concentrate. However Greenwood et al. (1997b) found weights 15% greater when bromegrass hay ground mixed was added in initiator concentrate. Beharka et al. (1998), found higher weaning weights (W60) when diets were added 25% alfalfa ground to initiator concentrate. The findings of this study show a positive trend on the CBW, and 60 d of age, on calves when compared to Group 1; nevertheless not statistically different (P > 0.05). These results based in limited numbers suggest an analysis of economic feasibility, so that it can be advise to dairy producers to make the best decision.

 Table 1. Composition of the diets on a dry basis in group 1 (Calves Starter), 2 (Calves Starter + 15 % ground alfalfa) and ground alfalfa

Measurement	Group 1	Group 2	Ground Alfalfa
Dry matter	92.54	92.47	89.56
Humidity	7.46	7.53	10.44
Crude Protein	14.78	15.46	18.91
Ash	4.21	8.85	10.83
Ether extract	1.34	1.90	1.44
Neutral detergent			
fiber (NDF)	11.83	15.63	44.04
Acid detergent			
fiber (ADF)	5.72	7.74	35.93

Table 2. Mean and standard de	eviation of birth weight (CBW), weight at 60 d (W60), average daily gain
(ADG) and total weight gain	(TWG) from birth to 60 d of age in the animals under study

Sex	Number	CBW	W60	ADG	GPT
	animals				
		Mean ± SD	Mean \pm SD	Mean ± SD	Mean ± SD
Females	26	37.8461±5.8288 a	61.6923±7.2375 a	0.4111±0.1093 ^a	23.8461±6.3415 a
Males	34	39.6764±7.3473 ^a	63.8823±7.9535 a	0.4173±0.0955 ^a	24.2058±5.5420 a
Total					
animals	60	60	60	60	60

^a Equal letters in columns indicate no significant difference (P>.05).

Table 3. Mean and standard deviation of birth weight (CBW), weight at 60 d (W60) from birth to 60 d of age in the animals under study

Treatment	Number of animals	CBW	W60
		Mean \pm SD	Mean \pm SD
Group1	31 (f=13and m=18)	40.8479 ± 1.2972^{a}	63.2794 ± 1.5496^{a}
Group2	29 (f=13 and m=16)	38.6940 ± 1.3166^{a}	61.9742 ± 1.5728^{a}
Total animals	60	60	60

^a Equal letters in columns indicate no significant difference (P>.05).

Table 4. Mean and standard deviation of the average daily gain (ADG) and total gain (TWG) from birth to 60 d of age in the animals under study

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Treatment	Number of animals	ADG	TWG
		Mean ± SD	Mean \pm SD
Group1	31 (f=19 and m=13)	0.3867 ± 0.0277^{a}	22.4314 ± 1.1448^{a}
Group2	29 (f=16 and m=14)	0.4013 ± 0.0200^{a}	23.2801 ± 1.1620^{a}
Total animals	60	60	60
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^a Equal letters in columns indicate no significant difference (P>.05).

Table 4 shows the mean values of ADG, and TWG from birth to 60 d of age in W60 in the animals under study (group 1 and 2). The ADG, was no different (P > 0.05) in the groups under study: 0.3867 ± 0.0277 and 0.4013 ± 0.0200 kg in group 1 and 2, respectively. A similar pattern was observed in the TW: $(22.4314 \pm 1.1448 \text{ and } 23.2801 \pm 1.1620 \text{ kg in})$ group 1 and 2), respectively. Coverdale et al. (2004) found lower ADG averages than the findings found in this study, when was added 7.5 and 15% bromegrass hay ground to the initiator concentrate, while in other studies (Montoro et al., and Castells et al., 2013) found higher average values than when compared to our findings. Although no statistical differences (P > 0.05) were found, in ADG, and TWG it was observed a slight positive trend towards the group that was fed calf starter, so further research is needed that level will be adequate for optimal results.

IMPLICATIONS

This study shows that the addition of forage and, the particle size can influence both the fed intake and behavior of calves. Forage (ground) of a consistent particle size can be used successfully in raising starter calves rations. The addition of ground alfalfa at initiator concentrate may be a way to reduce feed costs in rearing calves, results at this study based in limited numbers, suggest make an economic analysis. Further work is needed to determine the optimal levels of forage, when fed raising dairy calves in starter diets.

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Influence of the energy supplementation on voluntary intake and digestibility of ryegrass (*Loium multiflorum L.*) hay fed to Holstein steers

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ABSTRACT: The objective of this study was to evaluate the influence of energy supplementation on total feed intake of dry matter and the digestive function of nutrients in steers fed on ryegrass hay. The influence of energy supplementation (ES) on voluntary feed intake (VFI) and nutrient digestibility of annual ryegrass (Lolium multiflorum L.) hay (ARG) fed to Holstein steers was evaluated. Four steers Holstein, 250±5 kg BW, with cannulas in rumen and proximal duodenum, received ad libitum ARG with the following levels of energy supplementation (ES) as percentage of BW: T1 = 0% (ES0), T2=0.4% (ES04), T3=0.8% (ES08) and T4=1.2% (ES12). Energy supplementation decreased (P <0.05; linear component) forage intake and its contribution as organic matter (OM), Neutral Detergent Fiber (NDF) and N. The addition of energy decreased the digestibility in rumen of the OM (P < 0.01; linear component) and NDF (P < 0.03; linear component). Although were not detected differences (P > 0.05) between treatments, relative to the control treatments, with supplementation were 87% more efficient in conversion of OM fermented to microbial N. Non significant differences (P > 0.05) were detected of treatments on the components of the ruminal contents or the maximum intake expected. The optimal level of energy supplementation ranked between 0.4 and 0.8% of live weight relative to the observed values on the fibrous fraction digestion, efficiency of microbial synthesis, digestion in the rumen and total digestibility of dry matter and energy.

Key words: energy supplementation, voluntary intake, digestibility, ryegrass, Holstein steers

INTRODUCTION

Beef production under grazing depends on the seasonal variations and forage availability. At Mexicali Valley are grazed around 4,300 ha⁻¹ (SAGARPA, 2013) of annual ryegrass (*Lolium multiflorum L.*) by fattening steers and breeding heifers (Rodríguez et al., 2000). However, towards the end of the grazing season the growing rate in animals it is decreases (Cervantes *et to the.*, 2000) because of the reduction in the total feed intake, digestibility of dry matter and energy density of fodder (War, 1997). In this region of the extreme weather temperatures during winter and summer, most of the studies related to ryegrass pasture have been focused towards total forage yield, changes in its chemical

composition and digestibility or its impact on average daily gain; however, there was no background on the influence of energy supplementation in the use of the fiber fraction and the voluntary forage intake. The objective of this study is to evaluate the influence of energy supplementation on total feed intake of dry matter and the digestive function of nutrients in steers fed on ryegrass hay.

MATERIALS AND METHODS

The study was carried out in the room of digestion and metabolism of ruminants of the Agricultural Research Institute of the University Autonomous University of Baja California, located at Ejido Nuevo León, B.C. Four steers Holstein (250 \pm 5 kg PV) enabled with cannulas type "T" in rumen and proximal duodenum (114 and 25 mm internal diameter), respectively. The animals were fed ad libitum diet of annual ryegrass hay to 0800 and 1600 hr. Energy supplement (80% wheat rolled, yellow fat 8.4%, cane molasses 7.5%, salt 1.1% and 2% limestone) was offered as a percentage of BW of the animal, in the treatments: T1 =ad libitum ryegrass hay, T2 = ad libitum ryegrass + 0.4% of energy supplement, T3 = ad libitum ryegrass hay + 0.8%energy supplement, T4 = ad libitum ryegrass hay + 1.2%energy supplement. Chromium oxide was assigned (0.3% of the total DMI) offered as a digesta marker.

Daily at 0800 intake level was set at 110% of the previous day, fed rejection was recorded daily and reassigned to avoid loss of marker. The experiment consisted of four 14-d experimental periods, first 10 d were adapting to the diet and day 11 to 14 for the collection of fecal, duodenum and rumen samples. In each period the total DM intake per animal was the average of the three days prior to sampling. The last day of each collection period, 4-h post-feeding, samples of individual liquid in rumen were taken to estimate the pH, the mix it in proportion 4:1 with m-phosphoric acid 25%, weight/volume, for analysis of volatile fatty acids (VFA), subsequently the rumen was evacuated wet-dry VAC to quantify the total DM and NDF content in rumen.

Samples of food, chyme, feces and rumen contents were subjected to all or part of the following analysis: dry matter, ash, N-NH₃, total N (AOAC, 2000), FDN (Goering and Van Soest, 1970), chromic oxide (Hill and Anderson, 1958), purines as estimators of bacterial N (Zinn and Owens, 1986) and volatile fatty acids in the ruminal fluid by gas chromatography.

RESULTS AND DISCUSSION

The influence of energy supplementation on the voluntary intake VFI and the flow of nutrients into the duodenum in annual ryegrass hay fed to Holstein steers are presented Table 1. There was a decrease (P < 0.05; linear component) in the total forage intake, organic matter MO, NDF and N. It may be attributable to the effect of substitution of supplement on the forage basal diet. This replacement reached almost 50% in animals receiving the highest energy supplementation (1.2% BW). Also as a result of the lower content of NDF in the supplement, it gradually decreased the total NDF consumption by animals. Branine and Galyean (1990) found a similar behavior in cows supplemented with rolling sorghum to graze winter wheat.

Table 2 shows the influence of treatments on the digestibility and rumen synthesis. The addition of energy decreased the digestibility in rumen of MO (P < 0.01; linear component) and NDF (P < 0.03; linear component). Several authors explain this response as a negative impact on cellulolytic fermentation causes the increased availability

of soluble carbohydrates in rumen. Nevertheless, the total N rumen digestion presented a turning point (P < 0.08, quadratic component) between 0.4 and 1.2% of supplementation levels, respectively. In reference to the Control treatment, supplemented treatments were 87% more efficient (P < 0.05) in the conversion of MO fermented to microbial N; however non differences (P > 0.05) were detected between them.

The influence of energy supplementation in Holstein steers on the kinetics, pH and rumen content, and the proportion of volatile fatty acids observed in Table 3. There was no effect (P > 0.05) of treatments on the components of ruminal contents or maximum consumption expected. Under the conditions of this experiment differences (P > 0.05) were found on the rate of passage and digestion of NDF in rumen. Varga and Prigge et al. (1982) observed no influence of the level of DM intake; however several authors (Van Soest, 1994) Okine and Mathison, 1991; Daddo and Allen, 1995) agreed that the digesta spends less time in the rumen when consumption increases. The animals who received the higher level of energy supplement showed the highest (P = 0.05) cubic component)

 Table 1. Influence of the energy supplementation on the consumption of nutrients in Holstein calves fed ad libitum with annual ryegrass hay

 Distant transmit

	1	Dietary tr		Effect ²				
Item	0.0	0.4	0.8	1.2	EE	L	Q	С
Intake, (g/d)								
DM								
Forage	6570 ^a	5738 ^{ab}	5316 ^b	3258°	987	0.001	0.080	0.176
Supplement	0	64	103	167	5	0.000	0.000	0.000
Total	6570 ^a	5802 ^{ab}	5420 ^b	3425°	988	0.001	0.080	0.185
OM								
Forage	5796 ^a	5061 ^{ab}	4692 ^{be}	2872 ^d	874	0.001	0.084	0180
Supplement	0	19.2	30.6	50.9	1.8	0.000	0.000	0.000
Total	5796 ^a	5081 ^{ab}	4722 ^b	2924 ^e	874	0.001	0.084	0.183
FDN								
Forage	2627 ^a	2308 ^{ab}	2124 ^b	1312 ^e	418	0.001	0,178	0.331
Supplement	0	6.42	10.34	16.69	0.47	0.000	0.000	0.000
Total	2627 ^a	2314 ^{ab}	2135 ^b	1329 ^c	418	0.001	0,178	0.334
Ν								
Forage	103.2 ^a	91.9 ^{ab}	82.9 ^b	53.4 ^c	16.2	0.001	0.085	0.299
Supplement	0	0.76	1.23	1.98	0.06	0.000	0.000	0.000
Total	103.2 ^a	92.7 ^{ab}	84.1 ^b	55.4°	16.2	0.001	0.085	0.311

^{a, b, c, a} Means in the same row with different superscripts are significantly different (P<0.05).

¹Treatments: 0.0 = control, 0.4 = 0.4% energetic supplementation of BW; 0.8 = 0.8% energetic supplementation of BW; 1.2 = 1.2% energetic supplementation of BW.

 $^{2}L = \text{linear}; Q = \text{quadratic}; C = \text{cubic}.$

DM: Dry matter, OM: organic matter, NDF: neutral detergent fiber, N: nitrogen in food.

	Ľ	Dietary t	reatmer	nt ¹		Effect	2	
Item	0.0	0.4	0.8	1.2	EE	L	Q	С
Ruminal digestio	n, %							
OM	68.1ª	61.1 ^{ac}	57.7 ^{bc}	51.5 ^b	2.5	0.003	0.867	0.564
NDF	81.7ª	$78.8^{\rm ac}$	79.3 ^{ac}	74.9 ^{bc}	2.4	0.028	0.612	0.321
N	81.6	93.2	83.0	72.4	6.5	0.170	0.081	0.418
Synthesis in rumen								
MOM ^e	515 ^{ab}	684 ^{bc}	379^{acd}	127 ^{bd}	125	0.073	0.020	0.367
Microbial efficiency ^f	12.4ª	22.2 ^b	21.3 ^b	26.1 ^b	2.83	0.003	0.276	0.128
N ^g Total tract digestion, %	0.67ª	0.79 ^{ac}	0.83 ^{bc}	1.00 ^d	0.05	0.001	0.696	0.331
DM	79.6 ^a	78.7 ^a	77.6 ^a	69.4 ^b	1.8	0.001	0.013	0.201
OM	80.7 ^a	79.7 ^a	78.4^{a}	69.0 ^b	2.1	0.001	0.023	0.260
NDF	78.4 ^a	78.2 ^a	76.6 ^a	69.6 ^b	2.7	0.002	0.036	0.504
Ν	76.4 ^a	73.8 ^a	72.6 ^a	62.4 ^b	3.1	0.001	0.029	0.145
DE, %	78.1 ^a	76.2 ^a	75.2 ^a	62.9 ^b	2.2	0.001	0.038	0.161
a, b, c, a Means in the different (P<0.05	ne same i).	row wi	ith diffe	rent sup	erscri	pts are s	ignifica	antly

Table 2. Influence of the energy supplementation on ruminal and total tract digestion in Holstein calves fed ad libitum with annual ryegrass hay

different (P < 0.05). ¹Treatments: 0.0 = control, 0.4 = 0.4% energetic supplementation of BW; 0.8 = 0.8% energetic supplementation of BW; 1.2 = 1.2% energetic supplementation of BW. ²L = linear; Q = quadratic; C = cubic. DM: dry matter, OM: organic matter, NDF: neutral detergent fiber, N: nitrogen in food, MOM: microbial organic matter. ^e Microbial organic matter = (DM reaching duodenum x % microbial N) / 10. ^f Microbial efficiency = microbial N g / kg of fermented OM. ^g Efficiency of the nitrogen = g of N not ammonia reaching duodenum / g nitrogen consumed.

proportion of propionate, but did not exist a consistent response to supplementation in the other treatments. Although was not detected (P > 0.05) differences of the levels of supplementation on rumen pH, values were permissible for a proper rumen fermentation, conversely to what was observed by Leventini et al. (1990) when barley was added as 10, 30 and 50% of daily intake of MS to a forage diet.

IMPLICATIONS

Under the conditions of this study, the higher level of energy supplementation decreased the total dry matter intake, without supplement compensate consumed total N, the DM in rumen digestion and total digestible energy consumed. In agreement to the findings observed on the digestion of the fibrous fraction, efficiency of microbial synthesis, digestion in the rumen and total digestibility of dry matter and energy, the optimal level of energy supplementation is between 0.4 and 0.8% of the live weight.

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	Dietary treatment ¹						Effect ²	
Item	0.0	0.4	0.8	1.2	EE	L	Q	С
Rumen contents								
Total, kg	30.6	28.0	30.5	28.8	2.9	0.651	0.819	0.216
Liquid, kg	26.2	24.0	25.6	24.5	2.4	0.53	0.674	0.258
Solids, kg	4.38	4.22	4.88	4.34	0.72	0,685	0.539	0.196
NDF, %	51.2	50.2	45.3	49.3	4.3	0.444	0.420	0.361
PDM, %	14.2	14.9	15.7	14.9	1.4	0.359	0.277	0.589
Kinetics in rumen								
Кр	0.023	0.028	0.028	0.048	0.009	0.074	0.377	0.486
Kd Maximum	0.104	0.110	0.125	0.090	0.029	0.690	0.198	0.396
intake, g/d	12272	13551	14152	12958	2507	0.756	0.557	0.893
Proportions of vola	atile fatty aci	ids						
Acetate	76.60	77.44	73.25	71.06	3.86	0.252	0.702	0.691
Propionate	21.32	13.29	14,58	23.24	4.17	0.684	0.053	0.910
Butyrate Acetate/propiona	2.08	3.59	1.62	5.69	1.81	0.296	0.493	0.264
te	4.3	4.2	3.5	3.6	0.89	0.480	0.961	0.717
nH	6.16	6.09	5.77	6.03	0.21	0.309	0.284	0.248

Table 3. Influence of the energy supplementation on the ruminal contents, kinetic in rumen, the proportion of volatile fatty acid and pH in Holstein calves feed ad libitum with annual ryegrass hay

¹Treatments: 0.0 = control, 0.4 = 0.4% energetic supplementation of BW; 0.8 = 0.8%

energetic supplementation of BW; 1.2 = 1.2% energetic supplementation of BW.

 ^{2}L = linear; Q = quadratic; C = cubic.

Kp: rate of passage, Kd: rate of digestion.

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Comparison of crossbred heifers mated naturally to Red Poll Bulls for production and growth traits

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ABSTRACT: Crossbred dams when mated at different ages and in different years by natural service to Red Poll (n = 415)were compared for calf birth weight (BW), weaning weight (WW), calving difficulty (CD) measured categorically and binomially, and calf survival (S). Crossbred cows were from Angus (A), Hereford (H), Pinzgauer (P), Brahman (B), Sahiwal, and Tarentaise (T) crosses. The analytical model included dam line (breed combination of dam) age of dam, sex and year as fixed effects, whereas julian birth weight was a (covariate). For analyses with back cross dams, covariates were included in the model for fractions of inheritance from each breed and fraction of complete heterosis of dams due to Bos taurus by Bos taurus, Bos taurus x Bos indicus, Bos indicus x Bos indicus crosses instead of breed combination of dam. Differences (P < 0.05) among crossbred dams were observed for birth weight, weaning weight, and calving difficulty. Birth and weaning weights were less in female calves than bulls calves. Crosses involving Brahman and Sahiwal cows had least calving difficulty. Heifer calves had less calving difficulty than bull calves. There were few differences (P > 0.05) among dam breeds for survival at birth or 3 days after birth.

Key words: breed differences, birth weight, weaning weight, calving difficulty, survival

INTRODUCTION

Increased attention has been placed on (BW) because it is the single most important factor associated with (CD), especially in 2 year old dams where a one pound (454 g) increase in (BW) results in a 2% CD (Ritchie et al., 1993). Birth weight is an effective correlated trait that can be used to reduce (CD) or (BW) will lead to higher post natal weight (Bennett and Gregory, 2001). Calving difficulty results in a major economic loss to beef producers (Deutcher, 1991). This loss is estimated to be 750 million dollars annually, nation wide. In such a way (CD) is becoming a greater concern for beef producers because of the increased emphasis on rapid growth rates, heavier weaning weights and improved cow efficiency. Schemes for simultaneously, (BW) and postnatal weight have been proposed (Dickerson et al., 1974; MacNeil et al., 1998). Heterosis achieved through continuous crossbreding can be used to increase weight of calf weaned per cow exposed to breeding by 20% (Gregory and Cundiff, 1990). The objective of this study was to compare groups of crossbred dams when mated by natural service to specific sire breeds for calf weights, calving difficuty and calf survival.

MATERIALS AND METHODS

This study used data that were part of a program to characterize a broad range of biological types of cattle as represented by breeds that differ widely in trais such as milk level, growth rate, carcass composition and mature size at Meat Animal Research Center, in Clay Center Nebraska (Gregory., 1993).

The cows used to initiate this phase of the experiment (Cycle III, phase 3) were originally produced by mating Hereford (H), Angus (A) cows to produce F_1 crosses from Hereford (HA) and Angus (AH), Pinzgauer (PH and PA), Brahman (BH and BA), Sahiwal (SH and SA) and Tarentaise (TH and TA) sires. These females produced 3-way cross progeny (Cycle III, phase 3) from matings to Red Poll sires to produce calves at 2 years of age.

The AI matings in (Cycle III, phase 4) provided progenie that were 0, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ Brahman and Sahiwal. These crossbred dams produced calves at 2 and 3 years of age from matings by natural service to Red Poll sires.

Management

Cows were maintained on improved cool season (brome) or warm season pastures and feed (grass and alfalfa) or silage during the Winter. Calving was in the Spring (March to April). At birth all calves were identified, weighed, dehorned (paste) and vaccinated against viral scours. Calves were weaned at approximately 200 day.

Data Collection

The traits analyzed in this study were BW, WW (adjusted to 200d of age), (CD) measured categorically, (CE) measured binomially, and survival at weaning, ar 3d, and birth, respectively. Calving difficulty was subjectively evaluated using descriptive scores (i.e., no difficulty, 2=Little difficulty by hand, 3=Little difficulty with jack, to 7= Caesarean birth presentation).

Calving ease was also analyzed binomially with score 1=ease (categorical, 1 and 2) and 0= not so ease (categorical, 3 to 6). Survival was not analyzed for calves resulting from crossbred cows mated to Red Poll and Longhorn bulls because nearly, all calves survived to weaning.

Analyses of Data

Separate Analyses for each trait used the Multiple Trait Derivative Free Restricted Maximum Likelihood (MTDFREML) program (Bodman et al., 1993). The analytical model included: dam line (breed combination of dam), sex, and year as fixed effects, whereas julian birth day was a (covariate). For analyses with back cross dams, covariates were included in the model for fractions of inheritance from each breed and fraction of complete heterosis of dams due to Bos taurus by Bos taurus, Bos taurus x Bos indicus, Bos indicus x Bos indicus crosses instead of breed combination of dam. Variance components due to dam and residual effects were jointly estimated except for the matings to Red Poll bulls to crossbred cows that calved only once at 2 years of age. Standard errors were used to test significance of differences among crossbred dam groups.

RESULTS AND DISCUSSION

 F_1 cross cows were mated to Red Poll bulls (n=415). Analyses of calves of F_1 crossbred cows to calve at 2 years of age to Red Poll bulls are summarized in Table 1. There were considerable differences (P<.05) in weights of calves born to cows of different dam groups. Crosses involving inheritance of PxH, PxA, TxH dams mated to Red Poll sires produced the heaviest calves at birth. Intermediate weights at birth involved calves from crossbred AxH and BxH heifers inheritance. The smallest calves at birth were for calves of SxA and SxH cows. Gregory et al. (1992) using same data also reported heaviest birth weights involving inheritance of crossbred Pinzgauer dams. Table 1. Also shows considerable differences among crossbred dam groups in weaning weights of calves. Weights of calves of BxA and BxH dams were heavier than calves from dams of the other eight crosses. Intermediate weights at weaning involved inheritance of crossbred dams TxH and SxH. The smallest calves at weaning involved inheritance of corossbred HxA and AxH dams. In a crossbreeding program involving inheritance of Bos taurus x Bos indicus, the design of a mating system is very important due to a noticeable difference in the performance of reciprocal cross (Brahman bulls mated to Bos taurus cows versus Bos taurus bulls on Brahman cows) calves for several economically traits (Thallman et al., 1993).

Table 1. shows that calves from crosses involving Brahman and Sahiwal cows had least (CD). Intermediate (CD) involved inheritance of crossbred AxH and y TxA. Crossbred dams HxA, PxH, and PxA had the most calving difficulty. Significantly (P<.01) less calving difficulty was expressed in female than in bull calves.

Most differences among crossbred dams were not significant (P>.05) for survival at weaning, 3d, and at birth; however calves of PxH, HxA, and PxA had the poorest survival. Gregory et al. (1991) reported large differences among breed groups in calving difficulty and survival independent of breed effects on birth weight.

Item	BW (lb)	WW (lb)	CD-C	CE	SWn	S-3d	S-Bth
Mean	72.9	430	2.14	1.69	.89	0.91	0.93
Breed of							
dam							
HxA	1.9	-49	1.40	35	07	03	05
AxH	3.7	-39	.38	15	.08	.02	.07
BxH	3.6	39	64	.18	.04	.03	.03
BxA	2.2	44	50	.13	.03	.02	.04
SxH	-4.0	12	48	.13	.06	.02	.04
SxA	-7.0	1	45	.13	.03	.02	.03
PxH	12.5	-1	1.23	33	11	02	07
PxA	8.3	-11	.88	22	02	03	02
TxH	7.7	15	.65	19	01	04	02
TxA	2.0	-6	.61	15	08	05	10
SE ^b	±2.2	±10	±.33	±.10	±.07	±.04	±.06
Sex							
Heifer	-3.2	-24	59	.16	.01	.02	.02
SE ^c	±0.9683	±4.2784	±.1470	±.0422	±.0298	±.0173	±.0250
JBD	0.1120	0.070	.0070	0030	.0012	0007	0002
SEd	±.0300	±0.1300	±.0040	±.0010	±.0009	±.0005	±.0009

 Table 1. Solutions from analyses of records of calves of crossbred heifers mated to unidentified Red Poll bulls to calve at 2 years of age

^a BW= Birth weight (lb), WW= Weaning weight (lb), Calving difficulty measured categorically, CE= Calving ease measured binomially, SW= Survival at weaning, S-3d Survival at 3d, S-BTH=Survival at birth.

^b H=Hereferod, A=Angus, B= Brahman, S=Sahiwal, P= Pinzgauer, T= Tarentaise; average standard error of difference between crossbred dam groups and Sahiwal by Angus cows.

^c Bulls minus heifers; standard error of the difference between bull and heifer calves.

^d Julian birth day; standard error of regression coefficient.

Table 2. Phenotypic	variances and fractions of variance due to individual effects of dams with	iin
crossbred groups m	ated naturally to Red Poll bulls	

Sire of	Age of	BW (lb)	WW (lb)	CD-C	CE	S-Wn	S-3d	S-Bth
breed	dam							
Fraction of		0.47	0.48	0.26	0.10	С	С	С
variance								
due to dam								
effects								
Red Poll	2							
Phenotypic								
Variance								
Red Poll	2	95	16.52	2.13	0.17	0.09	0.03	0.07

^a BW= Birth weight (lb), WW= Weaning weight (lb), Calving difficulty measured categorically, CE= Calving ease measured binomially, SW= Survival at weaning, S-3d Survival at 3d, S-BTH=Survival at birth.

^b Estimates were not obtained because mean survival was nearly 100%.

Variance Due to Dams Within Dam Breed

Table 2 lists phenotypic variances and fractions due to individual effects of dams within breed of dam groups. As shown, variation due to dam effects was important for growth traits as birth and weaning weights, respectively. Table 2 also shows the phenotypic variance values for (BW) and (WW), corresponded to crossbred dams mated naturally to Red Poll bulls to calve at 2 years of age, respectively.

Table 2 also shows that phenotypic variance values for survival at weaning, at 3day, and survival at birth, corresponded to analyses of crossbred dams mated naturally to Red Poll bulls to calve a 2 years of age. There was not much variation among dams for survival probably because of uniformly high survival rates. Because nearly 100%, all animals survived, survival was not analyzed.

IMPLICATIONS

Producers must consider birth weight and calving difficulty as important traits in their breeding programs. These results show large differences among crossbred dams in calving difficulty. Calves from 2-year old HxA, PxH, PxA, and TxH crossbred dams, had more calving difficulty. The heaviest calves at birth were from PxH, PxA, and TxH dams. Lowest birth weights involved calves from SxA and SxH dams. Crossbred dams BxA and BxH produced the highest calves at weaning with low birth weights but also less calving difficulty.

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Weight, body condition and pospartum ovarian resumption in holstein friesian cows

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ABSTRACT: To evaluate the postpartum (pp) ovarian resumption, condition and body weight in dairy, 46 Holstein Friesian cows were used. Body condition (BC), weight at calving, 25 and 50 pp days was evaluated. Data were analyzed by ANOVA. The effect of the number of calving and BC on pp upon the cyclic ovarian reactivation was determined with a correlation analysis. The BC was: $3.2 \pm .45$, $3.1 \pm .34$ and 3.0± .31 at calving, 25 and 50 pp days, respectively. Weight at calving was 538.4 ± 72.8 kg, 25 pp days 508 ± 68.1 kg and 50 pp days, 503 ± 81.8 kg. 19.6% of the cows had dystocia first grade, 8.6% retained fetal membranes (RMF). The interval from calving to first service was 62.7 ± 29.2 days. There was 1.5 and 1.8 ng/ml (P > 0.05) of progesterone (P_A) at 30 and 50 pp days, respectively. At 30 d pp 45.7% of cows had >1 ng/ ml of P_{42} of these 24% continued with levels >1ng/ml. At 50 pp days 54.3% had >1 ng/ml P_{A} . At 50 pp days, only 20% of cows without ovarian activity remained.

Keys words: body condition, dairy cattle, postpartum period, ovarian resumption, progesterone, reproductive parameters

INTRODUCTION

During the last decades the relentless increase in milk production per cow has decreased reproductive efficiency prolonging reproductive parameters, because a regular ovarian activity was not present during the postpartum (pp), influencing the profitability of dairy farming (Espinosa, 2010). Milk production depends largely on the ability of the reproductive performance of the cows, since the lactation cycle is restarted at the end of gestation. The challenge for the dairy industry is the high level of production without affecting reproductive parameters. Reproductive efficiency is multifactorial and dependent of physiological changes, nutrition, genetics and biological factors such as general health and management of animals. Reproductive processes are modulated by endocrine and nutritional factors and environmental (Diaz, 2011). The reproductive physiology of dairy cows has changed after an intensive selection of over 50 years, focused on achieving high milk production, which may explain the decrease in reproductive efficiency (Córdova and Pérez, 2005). Reproductive disorders that occur in dairy cows affect herd profitability. The immediate postpartum period, also known as postpartum or period pp in dairy cattle, direct impact on subsequent productive and reproductive cycles, and the latter is measured by reproductive parameters, including calving interval is considered as one of the most important. Prolonged lactation decreased milk production in subsequent lactations and involves increased feed costs and keeping cows in unproductive periods. Furthermore, when short periods (<12 months) are given, these prevent the maximum recovery and repair of glandular tissue of the udder to initiate new lactation, which involves significant production losses. The same applies to other organs involved in reproduction of animals, because not properly recover to start a new pregnancy. In breeding programs, the flaws in the design caused by errors in detection of estrus, nutritional, environmental and infectious factors, generate economic losses due to calving interval is prolonged, resulting in an increase in days open, maintenance cows with low productivity, costs semen and additional professional services. The aim of this study was to evaluate the weight and body condition on ovarian resumption and days open in Holstein-Friesian cows.

MATERIALS AND METHODS

The work was conducted August 2011 to June 2012 at the Faculty of Veterinary Medicine of the Universidad Juarez del Estado de Durango, located at a latitude of 24 ° 40 '00 " west longitude 104 ° 40' 00" and 1890 m. The climate is BS1 (w) (e) dry temperate, with rainfall during the summer and average rainfall of 450 mm. The average temperature is 17.5 °C (INIFAP, 2011). 46 Holstein Freisian cows of different age and number of calving were used. Each cow days was observed before and at calving. Type of calving, expulsion of the fetal membranes, BC (Edmonson et al., 1989), and weight at calving, 25 and 50 pp days was evaluated. Were taken blood samples from coccygeal vein on 25 and 50 pp days, which were centrifuged at 3000 rpm/10 min. The serum was separated in 1.5 ml Eppendorf vials previously identified and stored at -20 °C until analysis. P₄ levels were determined by enzyme immunoassay (Pinzon, 2005). Data were analyzed by ANOVA (SPSS, 10). The effect of the number of calving and BC on pp upon the cyclic ovarian reactivation was determined with a correlation analysis.

RESULTS AND DISCUSSION

Cows averaged 2.6 ± 1.5 calving, the age of the animals was 4.2 ± 2 years. The 80.4% (37/46) of calving were normal

and 19% with first-degree dystocia (9/46). The 33% of difficult calving were twin, and of these, 33% occurred in heifers and 33% were caused by age. The average weight of calves at birth was: 40.4 ± 7.1 kg, weight by sex was 37.2 ± 5.3 and 44.0 ± 7.2 kg in females and males, respectively. There was an 8.6% (4/46) of retained fetal membranes (RMF); 75% of these occurred in cows with more than four calving.

The average weight of the calves of cows had RMF was 46.25 kg and 75% of these were females. 45.4% of the cows had their first estrus between 30 to 60 pp days. The first service pp was provided to 62.7 ± 29.2 days. The P₄ levels during the pp were 1.5 and 1.8 ng / ml (P > 0.05) at 30 and 50 pp days, respectively. At 30 pp days, 45.7% of the cows had >1 ng /ml of P₄, of these 24% continued P₄ levels >1 ng / ml at 50 pp days. The 58.7% of the cows resumed ovarian activity in one of the periods evaluated. While 21% was CL presents 30 to 50 pp days, 20% of the animals remained inactive after the evaluation period.

The correlations between the levels of P_4 and BC at calving are in Table 1. The uncomplicated calving is common in most cases of animals, however, recorded a 5% dystocia (Lopez et al., 2003); Villaseñor et al., (2010) 7%. The RMF is observed more frequently in dairy cattle, expulsion should occur within 12 h after birth (Manspeake, 2000), Cordova and

Pérez, (2005), consider that more than 6 h is RMF; Drillich et al., (2003) 12-24 h after the fetus expulsion. In this work were 8.6% of RMF, which is not considered problem. Grigera and Bargo, (2005) believe the calving BC is important (optimal 3.5) and cows should not lose more than one point the first 60 pp days. Thus, the BC is within the normal range, 3.4 to calving and 50 pp days, the BC average was 3.

The time from calving to first ovulation, depends on several factors: breed, nutrition, milk production, season, etc. Marquez (2003) reports that the first ovulation occurs after the lowest level of negative energy balance between (NEB) 17 to 42 pp days, but can occur 15 pp days, without estrous signs (80%) and one estrus at 30 pp days with the presence of a CL in the 30% of the cows (Cano, 2007); NEB is the main metabolic problem to appropriate LH release to ensure first ovulation pp. In this case, 76% of the cows presented ovarian activity at least once during the 50 pp days, in this study. The proportion of dystocia observed is normal, 66% of these cases were presented in heifers and cows with twin births. Difficult calving not always preceded RMF.

IMPLICATIONS

The parameters measured in dairy cows generally were altered. Regarding the resumption of cyclic activity, there is a good performance which is evidenced by the levels of P_4

	P.C.	P.C.	P.C.	D	D.
	BC	BC	BC	P4	P4
	calving	25 pp days	50 pp days	30 pp days	50 pp days
BC	1.0	.575**	.45**	.17	.17
calving					
		.00	.00	.25	.24
BC	.57**	1.0	.61**	.22	.15
25 pp days					
	.00		.00	.13	.31
BC	.45**	.61**	1.0	10	.32*
50 pp days					
	.00	.00		.49	.03
P4	.17	.22	10	1.0	16
30 nn days					
50 pp days	.25	.13	.49		.27
Р.	17	15	32*	- 16	1.0
50 pp days			10.40		
50 pp days	.24	.31	.03	.27	
		1.51	.00		•

Table 1. Correlation between body condition (BC) and progesterone (P₄) concentrations at 30 and 50 d postpartum (pp) in dairy cows

** Highly significant correlation (P < 0.01)

* Significant correlation (P < 0.05)

pp = postpartum period BC = body condition $P_4 = progesterone$

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>1 ng/ml at 30 and 50 pp days in 54.3% of cows, indicating that track more necessary considering the BC can open days shorten and regularize calving intervals in at least 60% of the animals.

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Retrospective analysis of the reproductive performance in dairy cattle in a semi-arid region of south central Durango México

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ABSTRACT: The aim to the study was to analyze the reproductive behavior of a herd of dairy cattle in a semiarid area. Records were collected on 228 cows of different ages in 2008 (n = 41), 2009 (n = 40) was obtained, 2010 (43), 2011(n = 52) and 2012 (n = 52). Data were analyzed by ANOVA; the difference between means was established with a Duncan test. Of calving occurred, 10.3% were double. Abortion rate was 5.9%. The interval from calving to first service was 66.2 \pm 2.0 days, the conception rate to first service was 35.9, 30.2, 30.8, 42.3 and 46.7 % in 2008, 2009, 2010, 2011 and 2012, respectively. On average the second service reached 64.1 %. It took 2.3 ± 1.5 services per conception, was 116.3 ± 4.7 days open and calving interval was 411.9 ± 8 days. Waste index was 23.4 % and it was similar between years (P >0.05). The cows culled for reason of age was 44% and 22% were culled due to reproductive performance. This work aims to address the deficit in milk production observed in Mexico which is related to poor reproductive performance, high levels of waste, cost and availability of replacement females, and a lack of decision-making tools to minimize production parameters in dairy herds.

Key words: dairy cattle, reproductive performance, semiarid region

INTRODUCTION

The dairy industry has evolved dramatically over the past decades (Nakada; 2006. Dochi *et al.*, 2010), there have been great advances in nutrition and reproductive management of herds. The reproductive performance of the animals the interaction of health, nutrition and genetics reflected. Maintaining a high reproductive efficiency of dairy herd is critical to achieve profitability in the short and medium term, allowing the increase in milk yield per day of herd life, the number of calves per cow and minimize costs.

To improve fertility in high producing dairy cows, it is necessary to assess their reproductive characteristics (Dochi *et al.*, 2010), have an index of pregnancy and calving to keep production constant and the number of female replacements. Low fertility negatively impacts milk production, reproductive management protocols and professional services increases, a larger number of animals discarded and more replacements are required. The calving interval has been its increment, a situation that is being observed in most countries, including developed (Nakada, 2006; Hausman, 2012). The pregnancy rate to first service fell from 9 to 15% in Japan, Holland, USA, etc. (Lilido, 2008, Nakao, 2008), the most cows require more than one service to pregnant (Quintela *et al.*, 2004).

The first ovulation postpartum (pp) occurs at 43 days or more and the number of cows were at 60 days pp has increased (Dobson *et al.*, 2007), a situation that can be inherent to physiological changes during lactation as well as influence of risk factors in each of the herds and to each particular cow. From 1994 to 2003, annual milk production per cow increased 15% in USA and 20% in Mexico (USDA, 2007), where there is a growing and unsatisfied demand bovine milk, so it is necessary to make efficient the reproductive behavior herds (Ramirez and Segura, 2009).

The problem is to identify and understand the factors limiting fertility and implement effective strategies and specific among herds (Dalton *et al.*, 2006). In medium and backyard herds, there is total or partial absence of records, making difficult decisions grounded in the productive and reproductive performance of each animal. The aim of the study was to evaluate the reproductive performance by time of year and identify reference parameters in dairy cattle in a semiarid area of northeastern Mexico.

MATERIALS AND METHODS

The work was done in the barn located on the Durango-El Mezquital road Km 11.5, latitude 23 ° 59 '12 "west longitude 104° 37' 38" and altitude of 1878 meters. Located between the parallels 26 ° 48 '22" and 22 ° 14' 22". The average temperature was 25.7 °C Maximum 31.1 ° and minimum 5 °C, precipitation of 500 and 700 mm, relative humidity 50% and irradiation of 250-270 w/m2 (CEVAG, 2012). The climate is BS1 (w) semi-temperate (Aguilar, 2001). Records of 228 cows of different number of births for 2008 (n = 41), 2009 (n = 40), 2010 (n = 43), 2011 (n = 52) and 2012 (n = 52) were used.

Nutritional management of the herd was as follows: 18% CP concentrate feed according to milk production (1:4), forage (alfalfa, oats and / or irrigated meadow according to availability and time of year) and silage corn. A mixture of mineralized salts (12% Ca, 12% P, 1.9% Mg, Se and 11 ppm vitamin E 200 IU / kg and trace minerals) was *at libitum*. Annually, animals are immunized against clostridial diseases

(Clostridium spp) and viral and bacterial respiratory (PI3, IBR, BVD, leptospirosis and Pasteurella spp), the herd is free of brucellosis and tuberculosis.

Replacement females are vaccinated only once against brucellosis (RB51). Cows have a dry 45 to 70 days period. The period previous calving care and later expulsion of fetal membranes watch them. Annually, animals are immunized against clostridial diseases (Clostridium spp) and viral and bacterial respiratory (PI3, IBR, BVD, leptospirosis and Pasteurella spp), the herd is free of brucellosis and tuberculosis.

Replacement females are vaccinated only once against brucellosis (RB51). Cows have a dry 45 to 70 days period. Period prior to calving and the expulsion of fetal membranes watch them. The course of uterine involution was monitored the first 20 d pp. Estrus was detected twice daily (am-pm) for 30 minutes each time, until the first service and then return to estrus was recorded.

The pregnancy diagnosis was performed 45 days after the last service by transrectal palpation.

Data were analyzed by ANOVA with a Student t test the difference between means was established by year and X^2 test was run for variables measured in% (SPSS, 2010).

RESULTS AND DISCUSSION

The number of services per season (Figure 1) did not differ (P > .05), the spring was different (P < .05) in occurrence of calving, registration was 19.7, 27.5, 28.3 and 24.5% for spring, summer, autumn and winter, respectively. 56% of births occurred in summer-autumn (Figure 1). As for the pups, 51.1 and 48.9% (P > 0.05) male and female, weighing 36.3 ± 5.0 and 41.6 ± 6.8 (p <0.05) for females and males, with an average of 39 ± 6.5 k.

Relation to reproductive behavior per year, shown in Table 1. The incidence of twin births per year was different (P > .05). The rate was 27.1% waste; the main cause was age (44%), 23% for infertility, 25.8% death in the stable, and 8% lower production. The cows on average were discarded at pp

 320 ± 247 days, with 7.5 ± 3.0 years and 3.1 ± 1.4 deliveries. Discarded animals, 32% were in the first 100 days of lactation, 4.8% from 101 to 200 days and 59.7% over 201 days.

The problems in milk production, it is clear our country imports 32% (SIAP, 2012) of dairy products to supply domestic demand, although the per capita consumption is still low (355 ml). This situation is exacerbated given the low reproductive performance and high levels of waste young adult cows and replacement animals for rearing stage.

Reproductive behavior determines production in dairy herds; this aspect is affected worldwide in the last 30 years as a result of selection pressure to increase production of milk per day / cow (Wiltbank, 2002; Diskin et al., 2006). The evidence suggests that reproductive physiology depends on the inherent high production and / or negative energy balance during the first phase of lactation changes, are increasingly. In this case the rate of culling cows was 27.1% in the last 5 years, whose causes were age (44%), 23% infertility, 25.8% death on the farm and 8% low production, the cows were discarded to 7.5 years with 4.5 calving. 32% were in the first stage of lactation, 4.8% from 101 to 200 days and 59.7% over 201 days.

Prolonged open days are related to the rate of disposal, as the second cause of waste is infertility. Reproductive efficiency in cattle measured by days to first service, days open, calving interval, services per conception, heat detection rate, pregnancy rate and birth rate. The parameters in this case are acceptable, although fertility rates to the first and second service are low. However, 90% is achieved with more than three services, hence the average services per conception is greater than that reported by Lilido, (2008); Christie et al., 2007) to $1.75. > 3 \ 1.62$ to $2.9 \ and \ 1.8 \ to \ 1.6$.

The higher proportion of males at birth decreases the availability of replacement females and selection pressure from birth to first lactation. Sartori et al., (2002); De Vries, (2005) reported that losses in dairy herds by fertility concept are increasing. Pregnancy rates decreased from 36% to 9 % and the open days increased from 112 to 166.



Figure 1. Services distribution during the year (left) and calving occurrence by season (right) in dairy cattle.

						Total
Item	2008	2009	2010	2011	2012	average
Number of calving	41	40	43	52	52	228
Twin calving (%)	0a	4.8b	11.4c	6.2b	5.2b	6.9
Abortions (%)	7.3	7.8	5.8	4.1	5.7	5.9
Culling (%)	21.9	20	23.2	26.9	25	23.4
		Ν	lean ± SE			
1st service pp (d)	64.2 ± 19.4	66.7 ± 5.5	67 ± 4.0	63.1 ± 2.3	70.2 ± 5.0	66.2 ± 2.0
Pregnancy 1st serv. (%)	35.8a	26.8b	31.4b	43.7a	47.3a	36.2
Pregnancy 2 nd serv. (%)	25.6a	39.02a	28.5a	18.7a	26.3a	30.4
Services/pregnancy	$2.3 \pm .21a$	$2.5\pm.24a$	$2.5 \pm .26a$	$2.5\pm.24a$	$2.1 \pm .22a$	$2.3\pm~1.5$
Days open (d)	$142\pm13.6a$	$101 \pm 7.7b$	$113.4 \pm 11b$	$135.5\pm7.5a$	$111.2\pm8.1b$	116.3 ± 4.7
Calving interval (d)	$439 \pm 15.8a$	$408 \pm 10.5 \mathrm{a}$	$356 \pm 8.0a$	$414 \pm 10.2a$	385 ±13.1a	411.9 ± 8.5

Table 1. Reproductive performance by year in Holstein-Frisian cows.

% Statistic Difference by X²

abc in rows indicate difference (p < 0.05)

Cyclicity, fertility and gestation had a seasonal pattern, the results indicate that the months in which there were higher rates of calving were those animals receiving effective service in autumn-winter and most deliveries are concentrated in summer-autumn.'

IMPLICATIONS

A detailed analysis of the information from each herd required behavior characterization is important for making decisions about nutritional management practices are concerned, in order to have acceptable parameters in the production cycle of cows.

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Effects of zilpaterol hydrochloride supplementation on growth performance, carcass characteristics and production economics of steers differing in biological type¹

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INTRODUCTION

ABSTRACT: The β–adrenergic agonist zilpaterol hydrochloride (ZH) affects skeletal muscle growth, but little is known if this response is influenced by differences in genetic background of cattle. The objective of this study was to determine the effects of ZH on growth, carcass characteristics and production economic responses of British (B) and British \times Continental (BC) steers. Steers of B (n=76) or BC (n=57) background were allocated to a randomized incomplete block design with a 2×2 treatment structure. Pens within each block \times biological type were randomly assigned to either ZH (8.3 mg/kg of DM; fed for the final 20 d before slaughter) or control (CON; 0 mg/kg ZH). Steers were ultrasounded before ZH inclusion and following withdrawal to determine the influence of ZH on change in longissimus muscle area (LMA), fat thickness and percent intramuscular fat (IMF). Carcass and feedlot performance data were collected and used to determine biological type and ZH effects on economic responses. The interaction of biological type × ZH supplementation did not influence (P > 0.05) any traits. Biological type did not influence (P > 0.05) change in ultrasound measurements during the ZH feeding period or any measures of feedlot performance. Carcasses from BC steers had larger (P < 0.05) LMA and improved (P < 0.05) YG while B steers had increased (P <0.01) marbling scores. British \times Continental steers produced a greater percentage (P < 0.05) of YG 2 and less YG 3 (P <0.05) than B steers. A greater proportion (P < 0.05) of B carcasses were classified as upper 2/3 Choice while a greater proportion of BC carcasses were included in the lower 1/3Choice designation. Carcass value per cwt was greater (P <0.01) for B compared with BC carcasses and other economic responses were similar (P > 0.05). Feeding ZH improved ADG (P < 0.05), YG (P < 0.05), and LMA (P < 0.001) and resulted in a greater proportion (P > 0.05) of carcasses classified as YG 2. Total carcass value was greater (P < 0.05) for ZH compared with CON. While CON had increased (P < 0.05) IMF during ZH feeding, this did not manifest into differences in Quality Grade. Biological type did not influence response to ZH in this experiment. Biological type influenced carcass grid premiums, but not overall carcass value. Treatment with ZH improved carcass value by increasing HCW.

Key words: Beef cattle, biological type, carcass, growth performance, zilpaterol hydrochloride

Inclusion of the β -adrenergic agonist zilpaterol hydrochloride (ZH, Zilmax®, Merck Animal Health, Summit, NJ) in beef finishing diets has demonstrated dramatic effects on skeletal muscle growth, shifting the composition of gain and resulting in improvements in ADG and G:F in the feedlot as well as increases in dressing percentage, HCW and cutability of the carcass (Delmore et al., 2010). Much work has focused on the effects of feeding ZH to a single 'type' of cattle but our understanding of feeding ZH to divergent 'biological types' commonly produced in the Northern Plains (British and British × Continental) is limited.

British and British \times Continental cattle have inherent differences in muscle and adipose composition that could result in differential responses to ZH. Therefore the objective of this study was to determine the effect of ZH on resultant growth performance, carcass characteristics and production economics of cattle with varied biological types.

MATERIALS AND METHODS

Animals.

The South Dakota State University (SDSU) Institutional Animal Care and Use Committee approved all procedures involving animals. Cows at the SDSU Cottonwood and Antelope Research stations, of primarily Angus genetics, were artificially inseminated to 1 of 2 bulls. Bulls were either 100% Angus or 50% Angus \times 50% Simmental from a common Angus sire (GAR Predestined). Clean-up bulls that were 100% Angus from the same common sire were used for 60 d post-AI. Therefore, all progeny were grandsons of a common sire that was a trait leader for carcass characteristics in the Angus breed. Steer progeny (n = 133) were transported after weaning to the University of Nebraska Panhandle Research Center feedlot. Steers were fed a common 60 roughage:40 concentrate (DM basis) backgrounding ration for 45 d prior to the start of the project. At the start of the trial, steers were fed a 45 roughage: 55 concentrate (DM basis) ration and were stepped up using three rations over a 63-d period to reach the final ration of 16 roughage:84 concentrate (DM basis). Steers remained on this ration until marketed

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(97 d or 126 d). Ration ingredients were alfalfa hay, corn silage, wet distillers grains plus solubles, dry rolled corn, and a supplement package (supplement was formulated to include 0.3% urea and to provide a dietary DM inclusion of 0.3% salt, 60 mg/kg of Fe, 40 mg/kg of Mg, 25 mg/kg of Mn, 10 mg/kg of Cu, 1 mg/kg of I, 0.15 mg/kg of Se, 1.5 IU/g of degradation resulting in increased LMA, decreased fat thickness and higher yielding carcasses (Scramlin et al., 2010). The lack of interaction in the present study indicated that even though the steers differed in genetic background, they responded similarly to ZH despite its more potent action.

Biological type main effect.

Biological type did not affect (P > 0.05) cumulative ADG, final BW, DMI, or G:F of steers (Table 1). The changes in ultrasound fat thickness, LMA, percent IMF, and ADG during the ZH feeding period were not different (P > 0.05) between the biological types of cattle investigated in this study. These results indicate B and BC cattle responded similarly in regard to deposition of the muscle and fat tissues evaluated over the ZH treatment period. Carcass evaluation revealed no difference (P > 0.05) in HCW between biological types; however BC had a larger (P < 0.05) LMA and improved (P < 0.05) YG compared with B carcasses. Carcasses produced by BC steers also tended to have reduced (P < 0.10)

fat thickness. Marbling score was increased (P < 0.01) in B carcasses compared with BC. British × Continental steers produced a greater proportion (P < 0.05) of YG 2 and less YG 3 (P < 0.05) than B steers (Table 2). However, a greater proportion (P < 0.05) of B carcasses were classified as upper 2/3 Choice and there was a trend for a greater proportion (P <0.15) of B carcasses grading Prime. There was no difference in the number of carcasses grading Select however there was an increase (P < 0.05) in the proportion of BC carcasses classified in the lower 1/3 of the Choice grade compared with B. Additionally, carcass value per cwt was greater (P < 0.01) for B than BC (Table 1) because of premiums on the grid for higher quality-grading carcasses. However, overall carcass value per hd was similar (P > 0.05) among B and BC as a result of greater value per cwt for B carcasses multiplied by numerically lower HCW for B than BC carcasses. Other economic parameters (FCOG or return on feed) were not influenced (P > 0.05) by biological type.

Zilpaterol treatment main effect.

Supplementation with ZH for 20 d prior to slaughter improved (P < 0.05 for the ZH treatment main effect) ADG during the ZH feeding period, did not affect (P > 0.05) overall ADG or DMI over the entire feeding period, but tended to improve (P < 0.10) overall G:F (Table 1). Treatment had

Table 1. Least squares means and SEM for performance trait responses to main effects of biological cattle type and zilpaterol hydrochloride (ZH)¹ supplementation²

any meter of a second sec								
	Biolog	tical type		ZH, mg/				
Item	British	British × Continental	P > F	0	8.3	SEM	P > F	
ADG, initial-final, kg	1.74 ± 0.075^{8}	1.76 ± 0.082	0.790	1.71	1.79	0.078	0.198	
Final BW, kg ³	556.7 ± 13.1	564.5 ± 14.1	0.451	555.2	565.9	13.5	0.281	
DMI, kg·hd ⁻¹ ·d ⁻¹	9.96 ± 0.579	10.06 ± 0.584	0.574	10.07	9.95	0.581	0.423	
G:F	0.178 ± 0.017	0.179 ± 0.017	0.826	0.174	0.183	0.017	0.089	
FT change ⁴ , cm	0.09 ± 0.037	0.07 ± 0.043	0.677	0.10	0.07	0.040	0.490	
LM area change4, cm2	7.3 ± 2.94	4.7 ± 3.18	0.298	3.9	8.1	3.03	0.092	
IMF change ⁴ , %	0.29 ± 0.188	0.39 ± 0.207	0.583	0.61	0.07	0.195	0.010	
ADG, ZH feeding								
period, kg	1.05 ± 0.087	1.11 ± 0.106	0.674	0.89	1.27	0.095	0.017	
HCW, kg	357.8 ± 8.1	362.1 ± 8.6	0.475	353.8	366.1	8.3	0.052	
LM area, cm ²	88.7 ± 1.83	92.5 ± 1.96	0.028	86.7	94.5	1.88	0.001	
Yield grade	2.95 ± 0.079	2.62 ± 0.091	0.020	2.96	2.60	0.086	0.014	
Fat thickness, cm	1.48 ± 0.063	1.30 ± 0.073	0.093	1.44	1.34	0.068	0.369	
Marbling Score5	592.5 ± 22.4	486.2 ± 24.4	0.001	544.4	534.4	23.1	0.581	
\$/cwt ⁶	210.43 ± 2.47	207.69 ± 2.50	0.009	209.03	209.09	2.48	0.938	
Carcass Value, \$/hd	1672.81 ± 50.50	1658.65 ± 52.30	0.617	1633.32	1698.13	51.19	0.038	
Feed COG7, S/kg	0.438 ± 0.038	0.427 ± 0.038	0.443	0.433	0.432	0.038	0.922	
Return on feed, S/hd	1043.32 ± 43.22	1047.73 ± 46.37	0.896	1029.44	1061.61	44.39	0.322	

¹Zilpaterol hydrochloride was administered during the final 20 d of the finishing period.

² The biological type × ZH interaction did not affect (P > 0.05) any performance traits.

³ Final BW were reduced by 4% as per standard industry shrink.

⁴ Change in ultrasound backfat thickness (FT), LM area, and intramuscular fat (IMF) during the 20-d ZH feeding period.

⁵400 = Slight⁰; 500 = Small⁰; 600 = Modest⁰

⁶Mean grid price per unit HCW.

7 Feed cost of gain

8 Reported as least squares means ± SEM.

Table 2. Least squares means \pm SEM for proportion of carcasses in each USDA yield grade (YG) and quality	grade in
response to main effects of biological cattle type and zilpaterol hydrochloride (ZH) ¹ supplementation ²	

	Biologic	Biological type			ZH, mg/kg of DM		
		British x					
Item	British	Continental	P > F	0	8.3	P > F	
Calculated USDA yield grad	le ³						
YG 2	0.2785 ± 0.060^{5}	0.5726 ± 0.074	0.016	0.3015 ± 0.066	0.5450 ± 0.070	0.034	
YG 3	0.6872 ± 0.074	0.4020 ± 0.062	0.019	0.6501 ± 0.081	0.4429 ± 0.085	0.059	
USDA quality grade4							
Prime	0.1224 ± 0.039	0.0351 ± 0.024	0.140	0.0837 ± 0.042	0.0526 ± 0.029	0.558	
Upper 3/3 Choice	0.6843 ± 0.053	0.3308 ± 0.063	0.006	0.5151 ± 0.064	0.5023 ± 0.069	0.895	
Lower 1/3 Choice	0.1292 ± 0.039	0.5297 ± 0.068	0.002	0.2527 ± 0.062	0.3308 ± 0.066	0.413	
Select	0.0496 ± 0.029	0.1232 ± 0.061	0.208	0.0789 ± 0.042	0.0788 ± 0.042	0.998	

¹Zilpaterol hydrochloride (ZH) was administered during the final 20 d of the finishing period.

² The biological type × ZH interaction did not affect (P > 0.05) USDA yield and quality grades.

³None of the carcasses graded YG 1 or 5, and only 2 Angus carcasses that did not receive ZH graded YG 4.

4 None of the carcasses graded Standard or lower.

⁵Reported as least squares means ± SEM.

no effect (P > 0.05) on any live BW recorded throughout this study (final BW in Table 1, other BW not shown). The difference between ultrasound measurements taken 4 d prior to ZH supplementation and on the day of slaughter revealed ZH treated cattle tended (P < 0.10) to gain more LMA during the treatment period compared with CON while CON cattle accumulated more (P < 0.01) intramuscular fat than ZH. The difference in fat thickness between the initial and final ultrasound was not different (P > 0.05) between treatments. Carcasses from ZH treated steers tended to have heavier (P <0.10) HCW than CON steers as well as increased (P < 0.001) LMA and improved (P < 0.05) YG. Despite greater accretion of IMF during the ZH feeding period, CON carcasses had similar (P > 0.05) marbling scores to ZH carcasses. In agreement with similar change between treatments in ultrasound fat thickness during the ZH feeding period, carcass backfat thickness was similar (P > 0.05) between B and BC. Additionally steers supplemented with ZH produced a greater (P < 0.05) percentage of YG 2 carcasses and tended to produce fewer (P < 0.10) YG 3 than CON fed steers (Table 2). Supplementation with ZH did not affect distribution of Quality Grades compared with CON carcasses. Steers supplemented with ZH produced carcasses with increased (P < 0.05) total value compared with CON (Table 1), however there were no differences between treatments for value per cwt, FCOG, or return on feed.

IMPLICATIONS

These data do not indicate that there was a differential response to ZH among the biological types of cattle included in this study. Responses among biological types were as expected for B vs. BC cattle types. The resultant economic effect was that grid premiums for higher-grading B cattle were offset by larger HCW for BC, leading to similar overall carcass values. Finally, ZH influenced growth and carcass traits as expected, adding value to carcasses primarily by increasing HCW.

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Effects of nutrition level during lactation and rearing periods on growth patterns, puberty onset and fertility rate in beef heifers

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ABSTRACT: This study analysed the effects of different nutrition levels from heifer birth to first Timed Artificial Insemination (TAI) at 15 months, on their growth patterns, puberty onset and fertility rate. Twenty-nine Parda de Montaña heifers, born in autumn, were assigned to two growth rates in the lactation period (0-6 months: 700 vs. 1000 g/d, to Low (L) and High (H), respectively) and in the rearing period (6-15 months: 700 vs. 1000 g/d, to Low (L) and High (H), respectively), resulting in 4 treatments: LL, LH, HL, HH. At 15 months of age an Ovsynch protocol with an intravaginal progesterone device was used to synchronize and breed the heifers. Weight was taken weekly from birth until breeding season was finished to study the evolution of weight and average daily gain (ADG) along the experiment. Heifers were bled weekly throughout the rearing period to determinate the onset at puberty through plasma progesterone concentration. Heifers' average daily gains were influenced by the lactation and the rearing nutrition levels, animals compensating the growth rates in the different phases. The age at onset of puberty was higher in the animals receiving the Lactation low nutrition level (P<0.01) and the Rearing low nutrition level (P<0.001). Heifers of all lots showed similar weights at onset of puberty (55% adult weight), conception age (16.4 months) and fertility rate (89%). It can be concluded that the advance of the first service from 21 to 15 months of age is possible in extensive systems of beef cattle, if growth rates of 1 kg/d during lactation or/and rearing are guaranteed. Additional research is needed to determinate the impacts on adult size and frequency of dystocia at first and subsequent calvings of early-bred heifers.

Key words: efficiency, management, performance, replacement, reproduction

INTRODUCTION

Development of heifers is a critical component of beef production enterprise (Grings *et al.*, 2007) because they are the future dams and the efficiency in beef production is based on dams' productivity. This productivity could be defined by reproductive performance, capacity to wean calves at relatively heavy weight and lifespan. Those aspects could be influenced by heifers' management from birth to first breeding. Therefore, heifers must be keep a specific replacement program to reach first calving at early age with sufficient development to avoid dystocia at first and subsequent calvings, and to ensure a long and efficient lifespan (Patterson *et al.*, 1992). However, in Spain sometimes due to extensification of beef cattle production (García-Martínez *et al.*, 2009) and other ones due to reduced size of farms, heifers do not get this differentiated management.

Growth rate, both before and after weaning, could influence in productive and reproductive performance such as weaning weight, adult body weight (BW), age at onset of puberty, fertility at first breeding, etc.

Onset of puberty is the essential first step in replacement heifers' process (Revilla *et al.*, 1992) and there are several major physiological, environmental, and managerial factors that can advance or delay the age at puberty Schillo *et al.* (1992) described that puberty is reached with a critical BW for each breed. The main objective should be that the most heifers reach that critical weight early to reach puberty and cycle regularly before the breeding season to improve conception rate at first artificial insemination (AI). Beef heifers that calve by 2 years of age have a greater lifetime production potential than heifers that calve at older ages (Patterson *et al.*, 1992). In order to achieve this goal, heifers must reach puberty before 12 to 13 months of age, conceive at 14 to 15 months of age, and calve at approximately 2 years of age (Schillo *et al.* 1992).

The objective of this experiment was to evaluate the effect of different feeding level from birth to first breeding (lactation (Lact, 0-6 months) and rearing (Rear, 6-15 months) period) on BW, average daily gain (ADG), onset of puberty and fertility after fixed-time artificial insemination (TAI) at 15 months of age.

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Management

The study was conducted in La Garcipollera Research Station, in the mountain area of the central Pyrenees (Spain $42^{\circ}37'_{N}$, $0^{\circ}30'_{W}$, 945 m a.s.l.). Twenty-nine Parda de Montaña (beef breed derived from Brown Swiss) heifer-calves born in autumn (average birth date 12 October) were used for this study. At calving cow-calf pairs were randomly assigned to one of the four management strategies in a 2 x 2 factorial experiment, combining two heifer growth rates in the lactation period (0-6 months: 700 *vs.* 1000 g/d to Low (L) *vs.* High (H), respectively) and two in the rearing period (6-15 months: 700 *vs.* 1000 g/d to Low (L) *vs.* High (H), respectively). Treatments were randomly balanced according to dam BW and body condition score (Lowman *et al.*, 1976), calf birth date and birth BW. Treatments: LL, LH, HL, HH.

Cow-calf pairs remained indoors throughout lactation in a loose-housing system with straw-bedded pens (one pen per treatment). Dams were fed in groups a total mixed ration to met maintenance requirements for energy and protein in a 580 kg beef cow producing approximately 9 kg of energy-corrected milk (NRC, 2000). Calves were kept in straw-bedded cubicles adjacent to their dams and allowed to suckle them twice daily for 30 min. In order to achieve the desired growth rates LactH heifers had free access to starter concentrate.

At six months (175 d, mean), calves were weaned and transported to CITA Research Station facilities (41°43'_N, 0°48'_W; 225 m a.s.l.) where the rearing period was carry out. In that period heifers were housed indoors in a loose-housing system with straw-bedded pens. Each group of heifers had a pen with fresh and clean water supplied *ad libitum*.

Throughout this period, to achieve desired rate gain, heifers were group-fed alfalfa hay *ad libitum* and 6g or 12g concentrate/kg BW to low daily gain (RearL) or high daily gain (RearH), respectively.

At 15 months was started 90 days of breeding season. An Ovsynch protocol with TAI was used to synchronized and breed the heifers. After TAI heifers were observed twice daily to detect the heat in the non-pregnant heifers and to proceed to insemination approximately 12 h after the detection of estrus.

First-service conception and overall pregnancy rates were determined by transrectal ultrasonography (Ultrasound: Aloka SSD-500V (Aloka, Japan), equipped with a lineararray, 7.5-MHz transducer) 31 d after TAI and 31 d after the conclusion of the breeding season.

The TAI day was taken to determine the age and body weight at first breeding. The number of AI required to get pregnant was recorded for every pregnant heifer and the last AI day was taken to determine the age and body weight at conception.

BW at first breeding and at conception was calculated as average weights at the TAI or last AI day, respectively, previous and subsequent weight.

First-service conception rate was determined as heifers pregnant at the TAI by total heifers. Conception rate was determined as total heifers pregnant in the breeding season by total heifers.

Feedstuff and intake

During lactation period dams were milked monthly to determine the calves' daily milk intake. In addition, LactH groups had free access to starter concentrate and their intake was group-recorded daily. The feed refusal was removed and weighed weekly.

Throughout rearing period alfalfa hay intake was grouprecorded weekly. The concentrate intake along this period was group-recorded daily and it was monthly adjusted by average group-weight.

Body Weight

Heifers were weighed, before morning feeding without prior deprivation of feed and water, once a week throughout the experiment. BW at experiment's key points was calculated as average of three consecutive weights. Heifers' average daily gain for each period was calculated by linear regression of weights against time.

Blood Sampling and Assays

Heifers were bled from coccygeal vein or artery weekly throughout the rearing period to determinate the onset at puberty through plasma progesterone concentration. Samples were taken into 9 ml heparinized tubes (Vacuette, Spain) and were centrifuged at 3500 min⁻¹ for 20 min at 4 °C immediately after collection and the plasma was harvested and frozen at -20°C until it was analyzed.

Plasma progesterone concentrations were measure using a commercial enzyme immunoassay kit (Ridgeway Science, UK).

Age at puberty was defined as 7 d before the date of collection of the first blood sample that contained >1 ng/ml of plasma progesterone. Puberty ADG was calculated by linear regression of birth-puberty weights against time.

We took the weaning day as date of onset at puberty for pubescent heifers at the beginning of rearing period. In addition, first day of synchronization protocol was taken as date of onset at puberty for prepuberal heifers at that moment.

Statistical Analysis

Data were analyzed as a completely random design using the GLM procedure of SAS (v.9.3; SAS Inst. Inc., Cary, NY), with nutrition levels at lactation and rearing phase and their interaction as fixed effects. Means were separated using LSMEANS procedure of SAS and *P*-values \pounds 0.05 were considered different. Fertility was analyzed using FREQ procedure of SAS (chi² test).

RESULTS AND DISCUSSION

Along lactation phase, with a similar milk intake (7.2 kg/d), provision of concentrate *ad libitum* (mean intake: 1.37 kg/d) allowed to LactH treatments reach greater ADG (1.063 vs. 0.672 kg/d to LactH and LactL, respectively, P<0.001). Both in the first trimester of this phase like, more intensively, in the second one due to the increased intake from 0.2 kg/d in the first month to 3.45 kg/d in the last one (Table 1). Similar quantities to those described by Blanco *et al.* (2008) in feeding calves under the same conditions.

During rearing period, the gains were influenced by feeding level at this period, with greater gain in heifers RearH than RearL (0.668 vs. 0.960 kg/d, respectively, P<0.001). This difference maybe was due to a lower concentrate intake (4.2 vs. 1.7 kg/d to RearH and RearL, respectively) that RearL treatments partially offset with a greater alfalfa hay intake (5.2 vs. 6.8 kg/d to RearH and RearL, respectively). In the same way, gains in this phase were influenced by the performance in the previous phase. LactL treatments had higher growth rate during rearing phase (0.870 vs. 0.759 kg/d to LactL and LactH, respectively, P<0.001) partially offsetting lower gain observed in the previous phase. This compensatory growth was more intensive in the first two trimester of rearing period and in the last one there were no differences between treatments. The concentrate and hay intake was similar for both treatments (2.9 and 6.1 kg/d of concentrate and hay, respectively). In view of these results would think of higher conversion efficiency in animals with compensatory growth (Hoch et al., 2005). Despite this higher growth, compensation was not complete, due to the marked difference weight at weaning (164 vs. 228 kg to LactL and LactH, respectively, P<0.001) (Figure 1), and the difference weights persisted between treatments at 15 months (414 vs. 455 kg LactL and LactH, respectively, P<0.01).

No differences were found in BW at onset of puberty between treatments (mean BW = 324 kg) (Table 2) confirming that puberty is reached with a critical BW for each breed (Schillo et al. 1992), in this case (Parda de Montaña breed)

Table 1. Gains of heifers from birth to first breeding according to management in the lactation and rearing periods.

LACT (0-6 months)	ths) Low		Hi	gh		1	P-value		
REAR (6-15 months)	Low (LL)	High (LH)	Low (HL)	High (HH)	SEM	LACT	REAR	LxR	
n	7	8	7	7					
ADG 0-3 mo	0.652 °	0.769 bc	0.875 ab	0.909 ^a	0.04	< 0.001	0.10	0.36	
ADG 3-6 mo	0.649 ^b	0.653 ^b	1.228 a	1.239 ^a	0.04	< 0.001	0.86	0.94	
ADG 6-9 mo	0.538 °	0.996 ^a	0.433 ^d	0.865 ^b	0.04	0.002	< 0.001	0.71	
ADG 9-12 mo	0.912 ^b	1.092 a	0.710 ^c	1.087 ^a	0.03	0.004	< 0.001	0.005	
ADG 12-15 mo	0.835 bc	0.947 ^a	0.761 ^c	0.937 ab	0.04	0.24	< 0.001	0.38	
ADG LACT	0.643 ^b	0.699 ^b	1.046 ^a	1.080 ^a	0.03	< 0.001	0.18	0.72	
ADG REAR	0.744 ^c	0.998 ^a	0.593 ^d	0.925 ^b	0.05	< 0.001	< 0.001	0.10	
ADG Birth-Puberty	0.680 ^c	0.863 ^b	0.833 ^b	1.085 ^a	0.02	< 0.001	< 0.001	0.14	

LACT: lactation period; REAR: rearing period; Low: 700 g/d; High: 1000g/d ADG = Average Daily Gain

a,b,c,d Means within a row with different superscripts differ (P < 0.05)



Figure 1. Weights of heifers throughout the experiment according to management in the lactation and rearing period.

L (Low gain weight = 700 g/d); H (High gain weight = 1000 g/d) ^{a,b,c,d}Means at a given age with different superscripts differ (P < 0.05)

LACT (0-6 months)	Lo	w	Hig	h		P-valu		
REAR (6-15 months)	Low (LL)	High (LH)	Low (HL)	High (HH)	SEM	LACT	REAR	LxR
Weight at puberty, kg	330.6	313.7	326.2	328.8	14.18	0.71	0.62	0.50
Age at puberty, mo	13.5 ^a	10.2 bc	11.3 ^b	9.2 °	0.52	0.005	< 0.001	0.26
% ABW at puberty	56	54	56	56	0.02	0.75	0.56	0.53
Weight at 1st AI, kg	388.1 ^d	464.5 ^b	424.8 °	513.1 ª	12.03	0.001	< 0.001	0.62
Age at 1st AI, mo	15.8	15.6	15.7	15.9	5.05	0.67	0.93	0.26
Conception BW, kg	382.3 ^d	486.2 ^b	435.1 °	530.2 ^a	11.36	< 0.001	< 0.001	0.71
Conception Age, mo	15.9	16.7	16.6	16.4	0.26	0.79	0.58	0.31
nº AI	1.20	2.25	2.00	1.67	0.45	0.99	0.48	0.16
Fertility at 1 st AI	4/7	2/8	4/7	3/7		0.58	0.19	
Fertility	5/7	8/8	7/7	6/7		0.58	0.50	

Table 2. Onset of puberty and fertility rate after fixed-time artificial insemination at 15 months of age according to heifer management in the lactation and rearing periods.

LACT: Lactation Period; REAR: Rearing Period; Low: 700 g/d; High: 1000g/d.

ABW: Adult Body Weight; AI: Artificial Insemination.

a,b,c,d Means within a row with different superscripts differ

around 328 kg BW (Olleta *et al.*, 1991). In the same way, taken 586 kg as adult BW in Parda de Montaña (Casasús *et al.*, 2002), heifers reached puberty with 55% of adult BW as Freetly *et al.* (2011) had suggested for a wide rank of breeds.

Age at puberty is correlated with ADG from birth (Patterson *et al.*, 1992) and a marked relationship between age at puberty and previous ADG was found. Heifers reached puberty younger if their ADG from birth was higher (r=-0.77, P<0.001).

Differences found in age at puberty between treatments were due to nutrition level during lactation phase (10.3 *vs.* 11.9 m at puberty to LactH and LactL, respectively) and, in contrast to earlier works (Wiltbank et al., 1966; Gasser *et al.*, 2006), further were due to nutrition level in rearing period (9.7 *vs.* 12.4 m at puberty to LactH and LactL, respectively).

90% of heifers were pubertal 60 days before the breeding season, thus achieving one of the principal objectives in replacements heifers, they have to reach puberty 30-45 days before the breeding season (Gasser, 2013) because the fertility can be increased until 21% from first to third estrus (Perry, 2012).

Other rule of thumb in replacement heifers is that their weight should be close to 60-65% of mature BW at first service. In this case, mean weight of heifers were above 65% of mature BW (381 kg) for all treatments.

When the synchronization protocol was started three heifers from LL treatment were prepubertal, despite having apparently optimal age and weight. However, the inclusion of a progestin in the synchronization protocol, got heifers' ovulation and they became pregnant at the first insemination as Perry (2012) described.

No differences were found in fertility between treatments at the first breeding, using TAI, nor at the end of breeding season getting only three non-pregnant heifers (one of them infertile). To reach this fertility were necessary 1.85 AI per heifer. Number of AI necessary to get pregnant was not influenced by management gotten by heifers along the experiment.

It can be concluded that, depending on the food availability, heifers would be able to compensate lower growth rates in previous phases. These preliminary results would confirm the feasibility of advancing the first service from 21 to 15 months of age in beef cattle. In extensive systems, using TAI at 15 months, it will be necessary to guarantee growth rates close to 1 kg/d during lactation or/and rearing, the first option consuming less amount of concentrate. Additional research is needed to determinate if no impact on adult size and dystocia frequency are registered at first and subsequent calvings

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Performance of Nellore steers in immunocastration and surgical castration supplemented on tropical pastures in Rondônia-Brazil

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ABSTRACT: The surgical method is the most widely used method for the castration of animals on cattle in Brazil, however, direct losses are observed by ranchers regarding infections, bleeding and even death in animals and, indirect reduction of weight gain due to post-operative stress. Immunocastration acts by temporarily suspending bull fertility, allows ranchers to get the benefits of castration in addition to advantages such as the handling of cattle promoting animal welfare, improvement of meat quality, control of sexual behavior and increased productivity. The study aimed to evaluate the performance of Nellore steers supplemented to grazing tropical pasture in the Amazon, subjected to two methods of castration. Cattle are grazed on 25 Ha, uniformly covered with Brachiaria brizantha, containing water and food trough with 40 cm linear spacing per animal. Thirty-six animals, Nellore breed, with initial age of 36.0 ± 0.71 months and body weight of 516.3 ± 4.56 kg were distributed in completely randomized design, in group of animals castrated surgically and via GnRH vaccine Bopriva® (Protocol with two doses) 90 days prior to slaughter, with 18 repetitions in each method. Live weight and average weight gain of animals were recorded at 30 d, 60 d and 90 days of the experiment and, after slaughter in the slaughterhouse, carcasses weight, subcutaneous fat thickness and the weight of hindquarter and forequarter carcass. The methods of castration did not influence (P > 0.05) in the final body weight $(559.8 \pm 6.17 \text{ vs. } 549.2 \pm 6.55 \text{ kg})$, weight gain (474) ± 0.052 vs. 322 ± 0.035 g) and carcass yield $(51.22 \pm 0.83$ vs. 51.55 ± 0.75 %) of Nellore steers created in tropical pasture to immunocastrated and surgically castrated, respectively. A longer vaccination protocol should be investigated to obtain answers on productive performance and expressive carcass characteristics of steers supplemented on pasture and subjected to the immunocastration method.

Key words: fat thickness, intact steers, yield carcass

INTRODUCTION

The synthesis of steroid hormones on carcass characteristics of intact bulls, provides advantages such as better performance in terms of weight gain and gain efficiency, greater efficiency in the rate of lipids and protein deposition that growth of 10 to 20% faster than castrated steers (Paulino and Cavali, 2010), production of carcasses with higher percentage of lean meat to the market (Rocha et al, 2012). However, intact bulls have undesirable characteristics to the consumer and the castration is characterized, especially for cattle created in extensive systems in the Amazon, as a requirement of slaughterhouses in order to get better quality carcasses, given by greater fat thickness deposition in carcass in order to avoid problems regarding the cold shortening, blackening of noble cuts by cold and exudative losses during the cooling of carcasses (Felício, 1993); as well as the higher pH and higher hindquarter of carcass yield.

Despite the protein-energy supplementation from animals created on pasture to reduce the age of slaughter and improve the carcass and meat characteristics, suppressing the negatives effects of stress caused by castration on weight gain (Rocha et al., 2012), traditionally makes use of crude surgical castration method in beef cattle (Hernandez et al., 2005). In order to inhibit the effect of androgenic hormones from puberty, especially by facilitating the management of intact bulls on the property, get animals less agitated and aggressive, reduce the sodomy, reduce the cost of labour and the movement of animals at pasture, and get higher prices for the best fat thickness, farmers use the method which often incurs post-surgical complications like bleeding, inflammation, infections and myiasis besides the stress cause reduction in consumption and weight gain.

The Bopriva® vaccine, alternative to surgical castration and regulated by the Ministério da Agricultura, Pecuária e Abastecimento consists of an analog of GnRF linked to a carrier protein that acts stimulating the animalys immune system to produce specific antibodies against gonadotropinreleasing factor (GnRF or GnRH) neutralizing them, resulting in the reduction of circulating sex hormones (LH and FSH) temporarily inhibiting the production of testosterone (Ribeiro et al., 2004), making it possible to take advantage of the performance characteristics of intact bulls, reduce sexual behaviors and aggressive temperament, promoting welfare, in addition to the improvement the quality of meat and increase the potential productivity. Facilitated by the injectable administration in the handling of routine on the farm the immunity was established a few days after the second dose and the efficiency of immunocastration varies depending on the interval between two or three applications, lasting up to five months; recommended in this way, the use of different protocols according to the production system (Roça et al., 2011).

The aim of this study was evaluate the productive performance of Nellore steers undergoing surgical castration and immunocastration methods, supplemented on tropical pastures.

MATERIAL AND METHODS

The research was conducted at the Olhos D'água Farm's and Federal University of Rondônia, Brazil, from May to Sept. 2012, were 90 day of experiments. Thirty-six intact Nellore bulls, with initial age of 36.0 ± 0.71 months and body weight of 516.3 ± 4.56 kg were used. The animals, from the creation system grazing *Brachiaria decumbens* Stapf., and receiving mineral supplement, were randomly distributed in two groups, being eighteen animals castrated by immunological method and eighteen animals castrated by surgical method, each animal was a replicate.

Thirty days before the initial weighing, all animals were subjected to the control of ecto and endo parasites, and the beginning of the experiment were individually identified by numbered earrings, submitted to the weighing, and animals immunocastrated, submitted to the first vaccination. The GnRH vaccine, Bopriva[®] (Pfizer Animal Health, Parkville, Australia), regulated by the Ministério da Agricultura, Pecuária e Abastecimento in Brazil, initiates immunity to gonadotropin-releasing factor (GnRF) in one to two weeks after the second dose of the vaccine. Each 1-mL dose contained 400 µg of a conjugate of modified GnRH peptide covalently linked to carrier protein, together with advasure, a low reactogenic aqueous adjuvant for use in cattle.

The vaccine was administered to cattle on the lateral aspect of the left side of the neck through a 12.5-mm, 16-gauge needle, using a safety vaccinator to prevent inadvertent self-administration. To 30 days, was taken the weighing of animals (**P30**), applied the second dose of the vaccine to immunocastrated and held surgical castration of animals the second batch. In the procedure of surgical castration has been cleaning the knife and scrotum with iodophor solution 1:250 and anesthesia of the spermatic cord with 2% lidocaine; longitudinal incisions was held to ± 8 cm in the bag, allowing exposure of the testicle testis and testicular inguino-ligament section.

Later the animals were allocated into a paddock with 25 hectare of area with *Brachiaria brizantha* cv. Marandu pasture, by the method of castration, to facilitate the management of animals, and supplemented daily with 800 g/ animal of protein-energetic supplement. Measurements were carried out at 60 d (**P60**) and 90 d (**P90**). The 90 days, the animals were transported with proper Animal Transportation Guide-GTA, for a commercial slaughterhouse to 165 km from the farm in trucks, respecting the density of 1.53 m² per animal, as suggested by the Farm Animal Welfare Council (FAWC). Kept in fasting of solids by 12:00 hours were slaughtered by cerebral concussion, followed jugular

and carotid section, following the Normative Instruction $n^{\circ}.3$ of Brazil, 2002. After the slaughter, the half-carcasses were identified, evaluated visually by scores of subcutaneous carcass fat (scores from 1 to 5 ranking the subcutaneous fat of absent to excessive) (Felicio, 1993), taken the weight and cooled to -20°C for 24 hours when, after boning, weights of hindquarter and forequarter of carcass are obtained.

The data were analyzed using the GLM procedure of SAS (2002) based on the following model: $Y_{ij} = m + T_i + e_{ij}$ where: $Y_{ij} =$ observation; m = general average; Ti = treatment effect i (surgical or immunocastrated); and eij = random error associated with each observation, with $\mu = 0$ and $\delta^2 e$.

RESULTS AND DISCUSSION

The methods of castration did not differ significantly (P > 0.05) to the body weight of steers to 30 days, 60 days and 90 days and for average daily gain (**ADG**), table 1. However, the immunocastrated steers showed the most about 10 kg and 152 g of body weight (**BW**) and ADG, respectively, compared to surgically castrated steers.

The levels of energy and nutrients used in nutritional plans may influence animal performance, so different answers will be occur in production systems to grazing and confined.

Amatayakul-Chantler et al (2012) studying Bos indicus crossbred bulls in feedlot observed differences in BW to 56 and 84 days to immunocastrated steers with two doses of anti-GnRH Bopriva[®] vaccine compared to intact bulls, as well as to the characteristics of carcass.

Hernandez et al. (2005) evaluating 4 doses of anti-LhRH, at 0, 141, 287 and 385 days in animals on pasture in Brazil did not observe differences for the performance of the animals which may be related to the interval between applications. Roça et al. (2011) evaluating Nellore steers created on pasture surgically castrated and immunocastrated with two or three doses of anti-GnRF (three and eight months of effect of castration, respectively) observed that immunocastrated steers with two or three doses of anti-GnRF presented more eye area loin without differences in weight gain when compared to surgically castrated steers and, the steers who received two doses presented higher weight of carcass and less subcutaneous fat thickness at the 12th rib that steers receiving three doses. The authors make use of the appropriate Protocol recommended for each breeding system.

About such caveats is possible to infer that the increase in performance and improvements in carcass characteristics of castrated steers could have occurred under three doses of the vaccine, whereas, animals created on pasture, more physically active, demand a long and intense Protocol of applications, due to grazing conditions provide lower weight gains when compared to confined conditions.

The hindquarter and forequarter weights of the carcass were similar (P > 0.05) to castration methods (Table 1), averaging 110.43 ± 1.58 kg and 134.15 ± 1.89 kg, respectively, showing the efficiency of immunocastration on hormonal control of development of hindquarter cut.

As for the types of cuts the forequarter cut appeared heavier in relation to hindquarter (P < 0,0001). For the

Table 1. Performance of Nellore steers submitted to immunocastration or surgical castration method, supplemented in tropical pasture in Rondonia - Brazil.

Characteristics	Immunocastration ¹	Surgical castration	CV (%)
Initial weight (kg)	517.14 ± 5.96	515.58 ± 6.96	6.63
Weight at 30 days (kg)	552.11± 10.2	541.12 ± 6.00	6.32
Weight at 60 days (kg)	540.44 ± 6.83	524.28 ± 5.61	6.00
Weight at 90 days (kg)	559.83 ± 6.17	549.17 ± 6.55	5.92
average daily gain (kg)	0.474 ± 0.052	0.322 ± 0.035	12.23
Hindquarter carcass (kg) ²	$112.12B \pm 1.67$	$108.74B \pm 1.33$	7.75
Forequarter carcass (kg) ²	$134.75A \pm 1.85$	$133.56A \pm 1.54$	6.76
Carcass weight (kg)	286.01 ± 3.83	279.75 ± 3.26	6.71
Subcutaneous fat scores (mm)	2.44 ± 0.10	2.66 ± 0.13	25.01
Yield carcass (%)	51.22 ± 0.83	51.55 ± 0.75	8.42

¹GnRH vaccine, Bopriva (Pfizer Animal Health, Parkville, Australia), was administered as a 1-mL dose via subcutaneous injection. ²Letras diferentes na mesma coluna diferem pelo teste F (P < 0.05)

industry, reducing the yield of the hindquarter in ratio to forequarter or special cut is economically desirable, as in this last meeting the noble cuts of carcasses with greater commercial value in the market.

There were not differences in the subcutaneous carcass fat (P > 0.05) between the methods of castration (table 1) due to the similar reduction of testosterone levels. The subcutaneous carcass fat were classified in score 2 considered scarce, with values below 3.0 mm of fat, minimum of fat required by slaughterhouses in order to preserve the carcasses and cuts during the cooling, ensuring the quality of the meat.

Ribeiro et al. (2004) achieved higher BW, ADG, carcass weight and muscle yield in intact bulls compared to immunocastrated and surgically castrated steers. Andreo et al. (2012) evaluating immunocastrated and intact animals reported greater weight gain, weight and yield carcass for the steers, except for subcutaneous fat thickness in immunocastrated steers. According to the authors, the higher income can be explained by greater body development (marketable cuts) of the intact bulls.

IMPLICATIONS

The survey results were consistent with the hypothesis of study and responsive to effect between castration methods for performance of Nellore steers supplemented in tropical pastures.

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Effects of nutrition level during lactation and rearing periods on growth patterns, puberty onset and fertility rate in beef heifers¹

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ABSTRACT: This study analysed the effects of different nutrition levels from heifer birth to first timed artificial insemination at 15 months, on their growth patterns, puberty onset and fertility rate. Twenty-nine Parda de Montaña heifers, born in autumn, were assigned to two planes of nutrition in the lactation period (0-6 months: 700 vs. 1000 g/d, to Low (L) and High (H), respectively) and in the rearing period (6-15 months: 700 vs. 1000 g/d, to Low (L) and High (H), respectively), resulting in 4 treatments: LL (n=7), LH (n=8), HL (n=7), HH (n=7). At 15 months of age an Ovsynch protocol with an intravaginal progesterone device was used to synchronize and breed the heifers. Weight was taken weekly from birth until breeding season was finished to study the evolution of weight and average daily gain (ADG) along the experiment. Heifers were bled weekly throughout the rearing period to determinate the onset at puberty through plasma progesterone concentration. Heifers' average daily gains were influenced by the lactation and the rearing nutrition levels. Also, heifers with low ADG in the lactation period had higher growth rate during the rearing phase (P < 0.001), partially counterbalancing the lower gains in the previous one. The age at onset of puberty was higher in the animals receiving the lactation low nutrition level (P < 0.01) and the rearing low nutrition level (P < 0.001). All treatments showed similar weights at onset of puberty (55% adult weight) and fertility rate (86%). However, heifers' conception age was higher to heifers with high ADG during rearing phase (P < 0.05) due to they needed more number of artificial inseminations to get pregnant (P < 0.05). It can be concluded that the advance of the first service from 21 to 15 months of age is possible in extensive systems of beef cattle, using an Ovsynch protocol with an intravaginal progesterone device at 15 months. Our results suggest that 700g/d gains from birth until breeding season are sufficient without negative impact in reproduction performance at first breeding season. Additional research is needed to determinate the impacts on adult size, productive and reproductive performance at first and subsequent calvings and lifespan of early-bred heifers.

Key words: efficiency, management, performance, replacement, reproduction

INTRODUCTION

Development of heifers is a critical component of the beef production enterprise (Grings et al., 2007), because they are the future dams and the efficiency in beef production is based on dam productivity. This productivity depends on reproductive performance, ability to wean heavy calves and long lifespan. These aspects could be influenced by heifers' management from birth to first breeding. Therefore, specific replacement programs have to be developed in order to reach first calving at early age with sufficient development to avoid dystocia at first and subsequent calvings, and to ensure a long and efficient lifespan (Patterson et al., 1992). In European mountain areas, however, due to the extensification of beef cattle production systems (García-Martínez et al., 2009) or to small farm size, heifers are often managed along with the rest of the herd.

Growth rate, both before and after weaning, could influence productive and reproductive performance (weaning weight, adult weight, age at onset of puberty, fertility rate at first breeding, etc.). Onset of puberty is the essential step in heifer development (Revilla et al., 1992), and there are several major physiological, environmental and management factors that can advance or delay the age at puberty. Schillo et al. (1992) described that puberty is reached at a critical body weight (BW) for each breed. The main objective should be that the most heifers reach that critical weight early, in order to reach puberty and cycle regularly before the breeding season and consequently improve conception rate at first artificial insemination (AI). Beef heifers that calve by 2 years of age have a greater lifetime production potential than heifers calving at older ages (Patterson et al., 1992). In order to achieve this goal, heifers must reach puberty before 12 to 13 months of age, conceive at 14 to 15 months of age, and calve at approximately 2 years of age (Schillo et al., 1992).

The objective of this experiment was to evaluate the effect of different feeding managements from birth to first breeding (lactation (Lact, 0-6 months) and rearing (Rear, 6-15 months) periods) on heifer BW, average daily gain (ADG), onset of puberty and fertility rate after Timed artificial insemination (TAI) at 15 months of age.

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Management

The study was conducted in La Garcipollera Research Station, in the mountain area of the central Pyrenees (Spain $42^{\circ}37^{\circ}N$, $0^{\circ}30^{\circ}W$, 945 m a.s.l.). Twenty-nine Parda de Montaña (beef breed derived from Brown Swiss) heifercalves born in autumn (average birth date 12 October) were used for this study. At calving, cow-calf pairs were randomly assigned to one of the four management strategies in a 2 x 2 factorial experiment, combining two heifer growth rates in the lactation period (Lact 0-6 months: 700 *vs*. 1000 g/d, to Low (L) and High (H), respectively) and two in the rearing period (Rear 6-15 months: 700 *vs*. 1000 g/d, to Low (L) and High (H), resulting in 4 treatments: LL (n=7), LH (n=8), HL (n=7), HH (n=7). Treatments were randomly balanced according to dam BW and body condition score (Lowman et al., 1976), calf birth date and birth BW.

Cow-calf pairs remained indoors throughout lactation in a loose-housing system with straw-bedded pens (one pen per treatment). Dams were fed in groups a total mixed ration to meet maintenance requirements for energy and protein in a 580 kg beef cow producing 9 kg of energy-corrected milk (NRC, 2000). Calves were kept in straw-bedded cubicles adjacent to their dams and allowed to suckle them twice daily for 30 min. In order to achieve the desired growth rates LactH heifers had free access to starter concentrate.

At six months (175 d, mean), calves were weaned and transported to CITA Research Station facilities (41°43'N, 0°48'W; 225 m a.s.l.) where the rearing period was carried out. Heifers were housed indoors in a loose-housing system with straw-bedded pens. Each group of heifers had a pen with fresh and clean water supplied *ad libitum*. Throughout this period, to achieve desired rate gain, heifers were group-fed alfalfa hay *ad libitum* and 6 or 12g concentrate/kg BW to have low (RearL) or high daily gain (RearH), respectively.

At 15 months, a 90-day breeding season started. An Ovsynch protocol with an intravaginal progesterone device was used to synchronize and breed the heifers. After TAI heifers were observed twice daily to detect heat in nonpregnant ones and to inseminate again approximately 12 h after the detection of estrus. First-service conception and overall pregnancy rates were determined by transrectal ultrasonography (Ultrasound: Aloka SSD-500V (Aloka, Japan), equipped with a linear-array, 7.5-MHz transducer) 31 d after TAI and 31 d after the end of the breeding season. The day of TAI was considered to determine the age and body weight at first breeding, and the last AI day was used to determine the age and body weight at conception. Firstservice conception rate was determined as heifers pregnant at the TAI by total number of heifers. Conception rate was determined as total heifers pregnant in the breeding season by total number of heifers. The number of AI required to get pregnant was recorded for every pregnant heifer.

Measurements

Feed Intake. During lactation period dams were milked monthly to determine the calves' daily milk intake. In

addition, LactH groups had free access to starter concentrate and their intake was group-recorded daily. The feed refusal was removed and weighed weekly. Throughout the rearing period alfalfa hay intake was group-recorded weekly by pen; concentrate intake was group-recorded daily and monthly adjusted by average group-weight.

Weight. Heifers were weighed before morning feeding without prior deprivation of feed and water, once a week throughout the experiment. BW at key points was calculated as the average of three consecutive weights. Average daily gain for each period was calculated by linear regression of weights against dates.

Blood Sampling and Assays. Heifers were bled from the coccygeal vein weekly throughout the rearing period to determine the onset at puberty by analysis of plasma progesterone concentration. Samples were taken into 9 ml heparinized tubes (Vacuette, Spain) and were centrifuged at 3500 min⁻¹ for 20 min at 4 °C immediately after collection, and the plasma was harvested and frozen at -20°C until it was analyzed. Plasma progesterone concentrations were measured using a commercial enzyme immunoassay kit (Ridgeway Science, UK). Age at puberty was defined as the date of collection of the first blood sample that contained >1 ng/ml of plasma progesterone. In addition, the first day of the synchronization protocol was taken as date of onset at puberty for prepuberal heifers at that moment. Birth to puberty ADG was calculated by linear regression of birth to puberty weights against dates.

Statistical Analysis

Data were analyzed as a completely random design using the GLM procedure of SAS (v.9.3; SAS Inst. Inc., Cary, NY, USA), with nutrition levels at lactation and rearing phase and their interaction as fixed effects. Means were separated using LSMEANS procedure of SAS and *P*-values < 0.05 were considered different. Fertility rate was analyzed using the FREQ procedure of SAS (chi² test).

RESULTS AND DISCUSSION

Along the lactation phase, with a similar milk intake from their dams (7.2 kg/d), the provision of concentrate *ad libitum* (mean intake 1.37 kg/d) allowed LactH treatments to reach greater ADG than their counterparts (1.063 vs. 0.672 kg/d in LactH and LactL, respectively, P<0.001). This occurred both in the first half of lactation and, more intensively, in the second one (Table 1), which was due to the increased concentrate intake from 0.2 kg/d in the first month to 3.45 kg/d in the sixth one. Intake was similar to that described by Blanco et al. (2008) in suckling calves under similar conditions.

During the rearing period, gains were influenced by feeding level in this phase, with greater gains in RearH than RearL heifers (0.960 vs. 0.668 kg/d, respectively, P < 0.001). This difference may be due to a lower concentrate intake (4.2 vs. 1.7 kg/d, to RearH and RearL, respectively), that RearL heifers partly offset with a greater alfalfa hay intake (5.2 vs. 6.8 kg/d, to RearH and RearL, respectively). In the same

Table 1. Gains of heifers from birth to first breeding according to management in the lactation and rearing periods

LACT (0-6 months)	L	DW	High			P-value		
REAR (6-15 months)	Low (LL)	High (LH)	Low (HL)	High (HH)	SEM	LACT	REAR	LxR
n	7	8	7	7				
ADG 0-3 mo	0.652 °	0.769 bc	0.875 ab	0.909 ^a	0.04	< 0.001	0.10	0.36
ADG 3-6 mo	0.649 ^b	0.653 ^b	1.228 ª	1.239 ^a	0.04	< 0.001	0.86	0.94
ADG 6-9 mo	0.538 °	0.996 ^a	0.433 ^d	0.865 ^b	0.04	0.002	< 0.001	0.71
ADG 9-12 mo	0.912 ^b	1.092 a	0.710 °	1.087 ^a	0.03	0.004	< 0.001	0.005
ADG 12-15 mo	0.835 bc	0.947 ^a	0.761 °	0.937 ^{ab}	0.04	0.24	< 0.001	0.38
ADG LACT	0.643 ^b	0.699 ^b	1.046 a	1.080 ^a	0.03	< 0.001	0.18	0.72
ADG REAR	0.744 °	0.998 ^a	0.593 ^d	0.925 ^b	0.05	< 0.001	< 0.001	0.10
ADG Birth-Puberty	0.680 °	0.863 ^b	0.833 ^b	1.085 ^a	0.02	< 0.001	< 0.001	0.14

LACT: lactation period; REAR: rearing period; Low: 700 g/d ADG; High: 1000g/d ADG ADG = Average Daily Gain

^{a,b,c,d}Means within a row with different superscripts differ (P < 0.05)

way, gains in this phase were influenced by performance in the previous phase. LactL treatments had higher growth rate during the rearing phase (0.870 vs. 0.759 kg/d, to LactL and LactH, respectively, P < 0.001), partially counterbalancing the lower gains in the previous one. This compensatory growth was more intensive in the first two trimesters of the rearing period, while in the last one differences between Lact treatments were not significant. The concentrate and hay intakes were similar for both treatments (2.9 and 6.1 kg/d of concentrate and hay, respectively). Consequently, feed conversion efficiency may have been higher in animals with compensatory growth (Hoch et al., 2005). Despite this higher growth, compensation was not complete, due to the large difference in weight at weaning (164 vs. 228 kg, in LactL and LactH, respectively, P < 0.001) (Figure 1), which persisted to some extent at 15 months (414 vs. 455 kg, in LactL and LactH, respectively, P < 0.01).

No differences were found in BW at onset of puberty between treatments (mean 324 kg) (Table 2), confirming that puberty is reached at a critical BW for each breed (Schillo et al. 1992), and corroborating previous results with this breed (Revilla et al., 1992). In the same way, considering 586 kg as adult BW of Parda de Montaña cows (Casasús et al., 2002), heifers reached puberty at 55% of adult BW, as Freetly et al. (2011) had suggested for a wide range of breeds. Age at puberty is correlated with ADG from birth (Patterson et al., 1992), so that faster-growing heifers reach puberty earlier. In our experiment, a marked negative relationship between age



Figure 1. Weights of heifers throughout the experiment according to management in the lactation and rearing period.

LACT (0-6 months)	Lo	w	Hig	h		P-value		
REAR (6-15 months)	Low (LL)	High (LH)	Low (HL)	High (HH)	SEM	LACT	REAR	LxR
Weight at puberty, kg	330.6	313.7	326.2	328.8	14.18	0.71	0.62	0.50
Age at puberty, mo	13.5 ^a	10.2 bc	11.3 ^b	9.2 °	0.52	0.005	< 0.001	0.26
% ABW at puberty	56	54	56	56	0.02	0.75	0.56	0.53
Weight at 1st AI, kg	388.1 ^d	464.5 ^b	424.8 °	513.1 ^a	12.03	0.001	< 0.001	0.62
Age at 1st AI, mo	15.8	15.6	15.7	15.9	0.15	0.67	0.93	0.26
Conception BW, kg	382.3 ^d	486.2 ^b	431.7 °	530.2 ^a	11.36	< 0.001	< 0.001	0.71
Conception Age, mo	15.9 ^b	16.7 ^a	15.9 ^b	16.4 ^{ab}	0.25	0.55	0.02	0.64
nº AI	1.20 ^b	2.25 ^a	1.33 ^b	1.67 ^a	0.30	0.49	0.04	0.27
Fertility at 1 st AI	4/7	2/8	4/7	3/7		0.58	0.19	
Fertility	5/7	8/8	6/7	6/7		0.94	0.25	

 Table 2. Onset of puberty and fertility rate after fixed-time artificial insemination at 15 months of age according to heifer management in the lactation and rearing periods

LACT: Lactation Period; REAR: Rearing Period; Low: 700 g/d; High: 1000g/d

ABW: Adult Body Weight; AI: Artificial Insemination

^{a,b,c,d}Means within a row with different superscripts differ ($P \le 0.05$)

at puberty and ADG from birth to onset of puberty was found (r=-0.77, P < 0.001). Differences in age at onset of puberty among treatments were due to nutrition level during lactation (10.3 vs. 11.9 months, to LactH and LactL, respectively, P < 0.01) and, in contrast to earlier works (Wiltbank et al., 1966; Gasser et al., 2006), also during the rearing period (9.7 vs. 12.4 months, to ReartH and RearL, respectively, P < 0.001).

Ninety per cent of heifers were pubertal 60 days before the breeding season, thus achieving one of the main objectives stated by replacement programs. Heifers should reach puberty 30-45 days before the breeding season (Gasser, 2013), because fertility rate can be increased up to 21% from the first to the third estrus (Perry, 2012). Besides, their weight should be close to 65% of mature BW at first service (Gasser, 2013), and in this case, mean weights of heifers for all treatments were above 65% of mature BW (381 kg). When the synchronization protocol was started three heifers (LL) were prepubertal, despite having apparently optimal age and weight. However, the inclusion of a progestin in the synchronization protocol, got heifers' ovulation and they became pregnant at the first insemination as Perry et al. (2012) described.

No differences were found in fertility rate between treatments at the first breeding, using TAI, nor at the end of breeding season getting only four non-pregnant heifers. Number of AI necessary to get pregnant was not influenced by management gotten by heifers in lactation phase (1.7 vs. 1.5 AI to LactL and LactH, respectively). However, that parameter was influenced by nutrition level in rearing phase; RearL heifers needed less number of AI to be pregnant than RearH ones (1.27 vs. 1.96, respectively, P < 0.05).

It can be concluded that, depending on food availability, heifers are able to compensate lower growth rates in previous phases and reach puberty at an early age. These results would confirm the feasibility of advancing the first service from 21 to 15 months of age in beef cattle. Our results suggest that 700g/d gains from birth until breeding season are sufficient without negative impact in reproduction performance at first breeding season in extensive systems using an Ovsynch protocol with an intravaginal progesterone device at 15. Additional research is needed to determinate the impacts on adult size, productive and reproductive performance at first and subsequent calvings and lifespan of early-bred heifers.

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Effect of delaying time of insemination in a fixed-timed AI protocol on pregnancy rates to sexed semen in postpartum beef cows¹

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INTRODUCTION

ABSTRACT: The objective of the current experiment was to determine if delaying time of insemination by 8 h in a fixed-time AI (FTAI) program would increase AI pregnancy rates to sexed semen. Over 3 breeding seasons, postpartum cows (n = 839) were estrous synchronized using the 5-d CO-Synch + CIDR system. Cows were given GnRH (100 µg i.m., Factrel) at time of insertion of a controlled internal drug releasing device (CIDR; Eazi-Breed CIDR). Five days later CIDR was removed and $PGF_{2\alpha}$ (25 mg i.m., Lutalyse) was given at CIDR removal and 8 h later. Estrus detection aids were applied at CIDR removal. Cows were inseminated with X-sorted or Y-sorted sexed semen at 72 h (NORM) or 80 h (DELAY) after CIDR removal, and GnRH was administered at AI. At insemination, estrus status was categorized as positive (YES), partial (QUES), unknown (NR) or negative (NO). Bulls were introduced to cows at 14 d and removed at 60 d after FTAI. Pregnancy diagnosis was performed by ultrasound at d 60 after FTAI and via palpation at 60 d after bull removal. There was no difference (P > 0.05) in pregnancy rates to sexed semen or final pregnancy rates between NORM and DELAY cows. Pregnancy to sexed semen averaged 36.3% whereas final pregnancy rates were 90.6%. More cows (P < 0.05) in the DELAY group expressed estrus before FTAI, but this increase did not alter pregnancy rates to sexed semen. Expression of estrus before FTAI increased (P < 0.02) pregnancy rates to sexed semen across treatments with differences being YES > QUES or NR > NO. Pregnancy rates tended (P < 0.07) to greater in Year 1 & 3 than in Year 2. We conclude that delaying insemination by 8 h in an FTAI protocol did not improve pregnancy rates to sexed semen despite more cows exhibiting estrus before FTAI. Further research into FTAI strategies for use with sexed semen is warranted.

Key words: beef cows, fixed-time AI, sexed semen.

The number of beef sires with commercially available sexed semen increased from 0 in 2007 to over 70 by 2011 (Hall, 2011). In contrast, sexed semen became commercially available to the dairy industry in 2003 with widespread use by 2006 (De Vries and Nebel, 2009). Sexed semen has multiple applications in the beef industry including development of maternal lines and increasing the percentage of steers produced (Hall and Glaze, 2014). However, decreased pregnancy rates to sexed semen compared to conventional semen are still a barrier to adoption of sexed semen in the beef industry.

When inseminated with sexed semen, beef cows and heifers have a 10% to 20% decrease in AI pregnancy rates compared to females inseminated with conventional semen (Seidel et al., 1999; Deutscher et al., 2002; Hall et al., 2010). This appears to be a function of damage to the sperm during sorting as increasing numbers of sexed sperm per straw only produces minimal increases in pregnancy rates to sexed semen (DeJarnette et al., 2007). Pregnancy rates to sexed semen are improved if females are inseminated 12-18 h after an express estrus or been in estrus prior to fixed-time AI (FTAI; Hall et al., 2010). Seidel and co-workers (1999) suggested that delaying the time of insemination after detected estrus to 18 to 24 h may improve pregnancy rates to sexed semen compared to insemination at the traditional 12 to 18 h after estrus.

Fixed-time AI combined with conventional semen results in pregnancy rates exceeding 60% in multiple studies (Patterson et al., 2011). Fixed-time AI is becoming the estrus synchronization and AI method of choice in beef cows. For sexed semen to be readily adopted, FTAI procedures that yield acceptable (> 50%) pregnancy rates need to be developed. The objectives of the present experiment were to determine if delaying insemination by 8 hours in an FTAI protocol would

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1) increase number of cows expressing estrus before FTAI and 2) improve pregnancy rates in cows inseminated with sexed semen.

MATERIALS AND METHODS

All procedures were approved by the University of Idaho Animal Care and Use Committee. Over a three year period, postpartum beef cows (n = 839; av. BW = 606 kg; av. BCS = 5.8) were stratified by age and days postpartum and randomly assigned to FTAI at 72 h (NORM; n = 460) or 80 h (DELAY; n = 379) after removal of a controlled internal drug release device (CIDR) in the 5-day CO-Synch + CIDR protocol (Johnson et al., 2013; Figure 1). Due to logistical constraints more cows were assigned to NORM in Year 1. In all other years, equal number of cows were assigned to NORM and DELAY. Synchronization and timing of insemination treatments were applied to individual animals with cow as the experimental unit.

At CIDR removal, heat detection patches (Estrotect, Denver, CO; Years 1 and 2) or tail paint (Year 3) were applied to cows. Estrus status was determined at time of FTAI. Cows were considered to be in estrus (YES; patch fully activated or no tail paint), probably in estrus (QUES; patch partially activated or partial tail paint), no record (NR; patch lost or failure to record), or not in estrus (NO).

Cows were inseminated by professional technicians with either Y-sorted or X-sorted semen. Time from CIDR removal to AI was recorded. Six to eight AI bulls were used per year. Within years, AI bulls were used equally across the two timing treatments. Different bulls were used during different years, so bull and year are confounded. Therefore, year and not bull was used for statistical analyses. Fourteen days after FTAI, cows were divided into three groups and placed with natural service sires for clean-up breeding. The bull to cow ratio was 1:40 to 1:50. Between 55 and 60 days after FTAI, pregnancy status and fetal age was determined by transrectal ultrasonography. Bulls were removed 60 days after FTAI and final pregnancy status was determined via palpation between 60 and 70 days after bull removal.

Results were analyzed by logistic regression using GENMOD procedures of SAS. Dependent variables were pregnancy rate to sexed semen and pregnancy rate overall. Models included treatment (NORM, DELAY), heat expression (YES, QUES, NR, NO), and year as well as their interactions. No interactions were significant (all P > 0.05), and they were eliminated from the models.

RESULTS

There was no difference (P > 0.7) in AI or final pregnancy rates to sexed semen between NORM and DELAY treatments in 5-day CO-Synch + CIDR program (Table 1). However, more DELAY cows were in estrus (P < 0.01) prior to FTAI compared to NORM cows (Table 2).

Estrus status at time of FTAI influenced AI (P < 0.002) and final (P < 0.02) pregnancy rates (Table 3). Cows in estrus (YES) before FTAI had greater (P < 0.02) AI pregnancy rates than cows marked as NR or QUES. The NR and QUES groups were more likely (P < 0.05) to become pregnant to AI compared to cows that did not exhibit any signs of estrus (NO). Cows in the NO group had diminished (P < 0.03) final pregnancy rates compared to cows in the other estrus groups. Year impacted (P < 0.002) final pregnancy rates with Year 1 final pregnancy (84.9%) rate less than Year 2 or 3 (92.6%)



NORM = TAI at 72 ± 2 hr after 1st PG with GnRH at timed AI. DELAY = TAI at 80 ± 2 hr after 1st PG with GnRH at timed AI.

GnRH = 100µg i.m. Factrel; PG = 25mg i.m. Lutalyse; CIDR = Eazi-Breed CIDR; 1.38 g progesterone

Figure 1. 5-day CIDR CO-Synch Protocol

5-day CO-Synch + CIDR[®]

Table 1. Impact of timing of AI in a fixed-time AI program on pregnancy rates to sexed semen¹.

	Percentage (Prop	oortion) Pregnant
Treatment	AI	Final
NORM	36.7 (169/460)	88.5 (407/460)
DELAY	35.6 (135/379)	92.0 (349/379)

All cows were synchronized with 5-day CO-Synch + CIDR protocol with AI at 72 (NORM) or 80 h (DELAY) after CIDR removal.

Table 2. Effect of timing of AI in a fixed time AI program on percentage of cows in various estrus status classifications¹.

		Estru	s status	
Treatment	Yes	NR	Ques	No
NORM	50.3ª	14.4	20.7 ^a	14.6 ^a
DELAY	61.7 ^b	18.2	11.9 ^b	8.1 ^b

¹All cows were synchronized with 5-day CO-Synch + CIDR protocol with AI at 72 (NORM) or 80 h (DELAY) after CIDR removal. Estrus status: YES = detection aid activated; QUES = detection aid partially activated; NR = detection aid lost or not recorded; NO = detection aid not activated.

^{a, b} Within columns, different superscripts indicated effect of treatment (P < 0.05).

Table 3. Effect of estrus status at time of AI in a fixed time AI program on pregnancy rate to sexed semen or final pregnancy rate¹.

		Estrus	s status	
Pregnant to	Yes	NR	Ques	No
AI	41.7ª	33.1 ^b	31.9 ^b	24.6°
Final	91.7ª	93.1ª	88.1 ^{a, b}	83.3 ^b

¹All cows were synchronized with 5-day CO-Synch + CIDR protocol with AI at 72 (NORM) or 80 h (DELAY) after CIDR removal. Estrus status: YES = detection aid activated; QUES = detection aid partially activated; NR = detection aid lost or not recorded; NO = detection aid not activated.

^{a, b, c} Within rows, different superscripts indicated effect of estrus status (P < 0.05).

and 94.2%, respectively). Year 2 AI pregnancy rate (31.3%) tended (P < 0.07) to be lower than Year 1 (37.0%) or Year 3 (40.7%) AI pregnancy rates.

DISCUSSION

Pregnancy rates to sexed semen were not affected by delaying insemination by 8 h in the FTAI protocol used in this study. In the present study, pregnancy rates to sexed semen were low, averaging 36%, and these low values are in agreement with other FTAI studies in beef cows (Rhinehart et al., 2011; Thomas et al., 2014). In contrast, pregnancy rates to sexed semen in FTAI programs have approached 50% in some studies (Hall et al., 2010; Sá Filho et al., 2012).

Compared to conventional semen in FTAI protocols, sexed semen yields a 10% to 20% decrease in pregnancy rates (Hall and Glaze, 2014). The decreased pregnancy rates are not merely a function of decreased sperm numbers in straws of sexed semen compared to conventional semen as increasing number of sexed sperm only enhanced pregnancy rates by approximately 5% to 7% (DeJarnette et al., 2007; Schenk et al., 2009). These sorting induced non-compensable traits include submicroscopic damage to sperm such as DNA damage and early capacitation (Carvalho et al., 2010). As a result, the lifespan of sorted sperm is decreased. Therefore, delaying insemination until closer to the time of ovulation may be one approach to improving pregnancy rates sexed semen (Seidel et al., 1999).

In the current study, delaying insemination by 8 h did not increase pregnancy rates to sexed semen. In contrast, when insemination was delayed 8 to 12 h in heifers inseminated based on detected estrus, pregnancy rates to sexed semen were improved (Seidel et al., 1999). One explanation is that the delay in timing of insemination did not optimize the time of insemination relative to ovulation. This is evidenced by the lack of increase in pregnancy rates to sexed semen in DELAY cows despite at 10% increase in cows expressing estrus before FTAI. In the present study and others (Hall et al., 2010; Meyer et al., 2012; Thomas et al., 2014), female cattle that exhibit estrus prior to insemination with sexed semen have greater pregnancy rates than females that fail to express estrus. With conventional semen, optimum time of insemination is 4 to 12 h after onset of estrus in dairy cows (Dransfield et al., 1998) and 8 to 24 h in beef heifers (Dorsey et al., 2011). It is generally accepted that the optimum time for insemination in beef cows is 12 to 16 h after onset of estrus. Some cows in the DELAY group may have been in estrus 24 to 36 hours before insemination in the present study. As a result, insemination would have occurred too close to or after ovulation which would result in conception failure.

Different bulls were used in different years in the present study; therefore, year and bull are confounded, and year was used for analyses. The decrease in pregnancy rates to sexed semen in Year 2 could be the result of a variety of factors including nutrition, weather, bull or a random effect. Within the present study, pregnancy rates to semen from different bulls varied from 13% to over 50% (data not shown). However, the low number of inseminations per bull would make detection of all but the biggest differences difficult.

At present, low pregnancy rates to sexed semen are a barrier to use of this technology in the commercial beef sector. In addition, the development of successful FTAI programs for use with conventional semen is increasing the use of AI in commercial cattle (Patterson et al., 2011). It is reasonable to assume that widespread adoption of sexed semen will be predicated on FTAI systems that produce pregnancy rates of 50% or better. Since cows expressing estrus before FTAI have greater pregnancy rates to sexed semen than cows not exhibiting estrus, development of FTAI protocols which maximize the percentage of females in estrus before FTAI may increase pregnancy rates to sexed semen. However,

as indicated in the present study, the synchrony of estrus among cows must be tight. Recently, an alternative to "pure" FTAI was developed by Missouri researchers (Thomas et al., 2014). Cows in estrus before FTAI are inseminated with sexed semen at the recommended time for the FTAI protocol, whereas non-responding cows are administered GnRH at the recommend time of FTAI, but inseminated 20 h later. This resulted in a significant increase in pregnancy rates in nonresponding cows.

IMPLICATIONS

Results from the present study indicate that delaying insemination in an FTAI program may not improve pregnancy rates to sexed semen. Programs that maximize the percentage of females exhibiting estrus before FTAI should improve pregnancy rates with sexed semen. When using a FTAI program with sexed semen, a viable alternative would be to inseminate all cows expressing estrus before FTAI with sexed semen and inseminate all non-responding cows with conventional semen.

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Effects of hCG administration in ewes post-mating on production of 3β-hydroxysteroid dehydrogenase, steroidogenic acute regulatory protein, and interferon-stimulated gene 15¹

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ABSTRACT: The objective was to determine if administration of human chorionic gonadotropin (hCG) on d 4 after mating would increase the synthesis of 3β-hydroxysteroid dehydrogenase (3β -HSD), steroidogenic acute regulatory protein (StAR), and interferon-stimulated gene 15 (ISG15) in ovine reproductive tissues. Ewes received 600 IU (4.8 mL) of hCG i.m. (n = 9) or saline (4.8 mL) for control ewes (n = 1)10) on d 4 post mating. Within each treatment, ewes were randomly assigned to 1 of 2 groups where half the ewes were euthanized 13 d post mating and the remaining ewes, that were confirmed pregnant, were euthanized 25 d post mating. Jugular blood samples were collected from all ewes starting on day of marking by ram (d 0 = d of P4 levels at 1.0 ng/mL or less) through their designated euthanasia day post breeding (d 13 or 25). On d 13 and 25 post mating, ovaries were collected, CL counted, and caruncular tissue was collected. On d 25 concepti were also collected and counted. Synthesis of 3β -HSD was not observed in caruncle tissue on either day tested. When the d 13 CL tissue was probed for 3B-HSD no difference (P = 0.17; data not shown) was detected between control and hCG treated ewes. However, d 25 CL tissue yielded a decrease (P < 0.05) in the protein production of 3β-HSD compared to control. Also on d 25 hCG treated ewes showed (P < 0.05) a decrease in StAR protein. No difference was found between treatments on d 13 (P = 0.66) and 25 (P =0.85) using caruncle tissue ipsilateral to a CL. No difference was observed between treatments in CL tissue on d 13 (P =0.33) nor d 25 when probing for ISG15; however d 25 had a tendency (P = 0.08) to have a lower expression in hCG treated ewes. Therefore, administration of hCG on d 4 post mating shows a decrease in the expression of StAR and 3β-HSD in CL tissue on d 25, but showed no effects within the CL on d 13 or caruncular tissues on either d 13 or 25 of pregnancy.

Key words: 3β-HSD, human chorionic gonadotropin, ISG15, progesterone, StAR

INTRODUCTION

Several reasons contribute to embryonic loss. However, a prominent theory is insufficient functionality of the CL to produce sufficient P4 levels to support conceptus survival and prime the uterus to become a responsive environment for the developing conceptus (Kittok et al., 1983). Insufficient luteal function (poor P4 synthesis and secretion) is believed to be an important contributor to reproductive failure in mammals. A CL producing suboptimal levels of P4 is most likely an indicator of the dam's inability to maintain and carry the pregnancy (Kittok et al., 1983).

Oxidation and isomerization of pregnenolone to progesterone is due to 3β -HSD (Caffrey et al., 1979). Stimulation of the CL by luteinizing hormone (LH) is essential for long term efficacy of P4 production through steroidogenesis including the maintenance of optimal amounts of StAR (Juengel et al., 1995). StAR is a protein on the external surface of the mitochondrial membrane that aids internalization of cholesterol for the purposes of steroid production (Stocco, 2001).

Maternal recognition of pregnancy is essential for establishment of pregnancy, and in ruminants the primary signal is secretion of interferon tau (IFN- τ) from the trophectoderm between d 10 and 21 of pregnancy. It exerts a paracrine effect on the uterine endometrium to abolish luteolytic mechanisms (Spencer et al., 1996) and stimulates expression of interferon stimulated genes such as ISG15 (Zhang and Zhang, 2011). The synthesis and secretion of ISG15 occurs in a manner similar to the peak production of IFN- τ (Austin et al., 1996). Mares induced to ovulate via 2500 IU of hCG had no ISG15 present during cyclicity and in pregnant mares ISG15 was found in superficial epithelium and endometrial stroma, with minimal staining for glandular epithelial cells (Klein et al., 2010). Intracellular proteins bound to ISG15 are thought to aid in the expression and conformation of several proteins found in the uterine lumen during the period of maternal recognition of pregnancy (Johnson et al., 1998).

Ewes administered hCG d 4 post-mating yielded greater serum P4 concentrations, greater numbers of CL per ewe, and a greater number of multiple concepti per ewe (Coleson et al., 2013). Our data supports previous reports demonstrating increased serum P4 concentrations in ewes administered hCG, which was due to the increased number of corpora lutea (Khan et al., 2009).

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We hypothesized that supplementation of hCG on d 4 after mating would 1) increase serum P4 concentrations by increasing the number of CL and 3β -HSD and StAR production, and 2) increase ISG15 production in reproductive tissues. The objective was to use western blot analysis to determine if administration of hCG on d 4 after mating would increase the synthesis of 3β -HSD, StAR, and ISG15 in ovine reproductive tissues.

MATERIALS AND METHODS

Animals and Treatment

All procedures involving animals were approved by the New Mexico State University Animal Care and Use Committee (IACUC #2012-018). Nineteen mixed-aged western whiteface ewes (BW = 70.5 ± 1.5 kg) were used. Ewes were randomly assigned to 1 of 2 treatments. Ewes received 600 IU (4.8 mL) of hCG (ProSpec-Tany Techno Gene Ltd., Ness Ziona, Israel, Cat #: hor-250) i.m. on d 4 (n = 9) or saline (4.8 mL) for control ewes (n = 10) post mating. Within each treatment, ewes were randomly assigned to 1 of 2 groups where half the ewes were euthanized 13 d post mating and the remaining ewes, that were confirmed pregnant, were euthanized between 22 and 25 d post mating. All other procedures were performed as previously described in the companion reading Coleson et al. (2013).

Protein Isolation

Protein was isolated from caruncle tissue ipsilateral to the CL (Car) and CL tissue by homogenizing 100 mg of tissue in 1 mL of radio-immunoprecipitation assay (RIPA) buffer (50 mM Tris (pH 7.4), 2 mM EDTA, 150 mM NaCl, 0.1% sodium dodecyl sulphate, 1.0% TritonX-100) supplemented with phosphatase and protease inhibitor cocktail tablets (Roche Applied Science, Germany). Samples were placed on ice for 15 min and then centrifuged at 12,000 × g for 10 min at 4°C and supernatants subsequently removed and stored at -80°C. Concentrations of protein were determined using BCA protein assay (Pierce, Rockford, IL).

Western Blot Analysis

Protein lysates were collected from ovine Car and CL tissues as described above. For 3β-HSD, equal amounts of protein (50 µg per well) were separated using 4-20% Mini-Protein TGX Precast gels (BioRad Laboratories, Hercules, CA) followed by transfer to methanol activated polyvinyl diflouride (PVDF) membranes for immunoblotting. After blocking in 5% non-fat milk made in Tris-buffered saline plus tween (TBS+T) (68.4 mM Tris Base, 10 mM NaCl, 0.10% tween-20, pH 7.6) for 1 h at room temperature, membranes were incubated with 3β -HSD primary antibody (sc-30820; Santa Cruz Biotechnology, Inc. Santa Cruz, CA) at 1:1,000 dilution in 5% non-fat milk made in TBS+T. A secondary donkey anti-goat IgG-horseradish peroxidase antibody (sc-2020) at a dilution of 1:10,000 was used. For StAR, equal amounts of protein (30 µg per well) were separated by SDS-PAGE using 10% polyacrylamide gels. Blocking was similar to 3β-HSD protocol for StAR. After blocking, StAR blots were incubated with the primary antibody (sc-25806) at 1:1000 dilution in 5% non-fat milk made in TBS+T. A secondary goat anti-rabbit IgG-horseradish peroxidase antibody (sc-2317) at a dilution of 1:20,000 was used. Blots probed for ISG15 were incubated with the primary antibody (#2743BC; Cell Signaling Technology, Danvers, MA 01923) at 1:500 dilution in 5% BSA made in TBS+T. A secondary donkey anti-rabbit IgG-horseradish peroxidase antibody (sc-2317) at a dilution of 1:10,000 was used. Beta actin protein was also determined to further demonstrate equal loading of protein. Anti-beta actin antibody (sc-47778) was used at a 1:1,000 dilution and an antimouse (sc-2005) secondary antibody at a dilution of 1:5,000 was used. Proteins were visualized by SuperSignal® West Dura Extended Duration Substrate kit (Thermo Scientific, Rockford, IL) and detected using the ChemiDoc [™] XRS and Image Lab Software Version 3 (BioRad Laboratories, Hercules, CA).

Statistical Analysis

The chemiluminescent signals for Western blots were quantified using the mean value (intensity) with the Image Lab software program for each band of interest and normalized by dividing by mean value (intensity) for beta actin. Significant changes were determined at P < 0.05 using an unpaired, two-tailed student's t-test on Prism (Version 5 from GraphPad Software, Inc.).

RESULTS

No expression of 3 β -HSD was found when probing the caruncle tissues. When the d 13 CL tissue was probed with 3 β -HSD again no difference (P = 0.17; data not shown) was seen between the control and hCG treated ewes. A decrease in 3BHSD protein (P < 0.05) was observed in CL from hCG treated ewes compared to control on d25 (Figure 1).

Ewes treated with hCG yielded a lower expression of StAR in CL than control ewes on d 25 (P < 0.05: Figure 2). However, no difference was noted between d 13 (P = 0.66) and 25 (P = 0.85) using Car tissue (data not shown). ISG15 showed no difference between treatments in CL tissue on d 13 (P = 0.33) nor d 25; however d 25 had a tendency (P = 0.08; data not shown) to express less in hCG treated ewes compared to control.

DISCUSSION

We previously reported increased serum P4 concentrations as a result of hCG treatment in ewes (Coleson 2013), yet the precise reason for the increase in P4 is not known. Data from this study did not support our hypothesis that supplementation with hCG on d 4 after mating increased 3β-HSD and StAR expressed. Also, the administration of hCG did not affect the amounts of ISG15 produced. Administration of hCG on d 4 increased serum P4 concentrations (Coleson et al., 2013), similar to other research conducted in our lab (Yates et al., 2009); (Coleson et al., 2013; Lankford et al., 2010; Richardson et al., 2011). In the companion study, ewes receiving hCG had an increased number of CL and increased serum P4 concentrations similar to data by Khan et al. (2009), in which ewes and ewe lambs were treated with 200 IU hCG.



Figure 1. Expression of protein for 3β -HSD was down regulated in hCG treated ewes (n = 6 for control ewes; n = 4 for hCG treated ewes; P < 0.05). Protein expression was measured using western blot procedures.

Granulosa cells collected from pigs administered hCG yielded an increase in the quantity of mRNA encoding for 3β -HSD (Chedrese et al., 1990). Our study demonstrated that 3β -HSD protein levels were lower expressed in hCG treated ewes but only on d 25 in CL tissues. Corpora lutea explants that were incubated with progesterone yielded a decrease in the enzyme rate of 3β -HSD, and was proposed that there was a product inhibition creating this decreased enzyme activity, so it is suggesting that there is a negative feedback inhibition happening when there is excessive P4 available (Caffrey et al., 1979).

Treatment of rat theca cells with hCG increased mRNA levels and protein production of StAR (Palaniappan and Menon, 2012). Our study demonstrated that StAR protein levels were lower expressed in hCG treated ewes but only on d 25 in CL tissues. Lea et. al. (2007) saw that the amounts of StAR, or any steroidogenic enzyme, was not affected with respect to the concentration of serum progesterone in ewes at d 81 of pregnancy. The decrease in StAR production in the current study could possibly be due to the elevated serum P4 concentrations within the hCG treated ewes, which could be acting upon a local negative feedback system.

We tried to indirectly determine if treated ewes secretion of IFN τ was altered by the treatment with hCG by probing for ISG15 in the uterus and CL. Nephew et al. (1994) found that administration of 100 IU of hCG to ewes on d 11.5 post mating resulted in longer concepti (3.5 ± 1.6 cm vs. 0.8 ± 0.5 cm; P < 0.05), and higher concentrations of IFN τ was found in uterine flushing. The ISG15 results did not support our hypothesis and we suggest administration of hCG does



Figure 2. Expression of protein for StAR was down regulated in hCG treated ewes (n = 6 for control ewes; n = 4 for hCG treated ewes; P < 0.05). Protein expression was measured using western blot procedures.

not alter the secretion of IFN τ by the conceptus, with respect to ISG15 synthesis in the caruncle and CL. Pregnant cows exhibit a higher expression of ISG15 compared to nonpregnant cows between d 18-20 of pregnancy (Green et al., 2010). Concentrations of mRNA for ISG15 is significantly (P < 0.05) increased in the endometrium from pregnant ewes on d 15 compared to non-pregnant ewes (Oliveira et al., 2008). Endometrium and CL also have increased concentrations of ISG15 during the maternal recognition of pregnancy phase in cattle and sheep (Hansen et al., 1997; Nitta et al., 2011).

We believe that the increased progesterone in the hCG treated ewes were due to the increased number of corpora lutea and does not appear to be due to the increase of 3β -HSD and StAR. We suspect that there is a local feedback mechanism in place for elevated P4 to down regulate the 3β -HSD and StAR at d 25 of pregnancy. Due to the high endogenous levels of progesterone the corpora lutea may be having a local negative feedback system down-regulating the production of progesterone.

IMPLICATIONS

Data reported herein does not support our hypothesis that administration of hCG to ewes on d 4 after mating increased serum P4 concentrations reported by Coleson et al. (2013) by increasing the amounts of 3 β -HSD and StAR expressed as well as an increased amount of ISG15 produced. Therefore, the greater serum P4 concentrations were due to a greater number of corpora lutea possessed by the hCG treated ewes.

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Effect of dried salmon silage on productive performance and gizzard health of broiler

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ABSTRACT: Farming of salmonids is very intensive in Chile, generating ecosystem disruptions by accumulation of mortality. These salmon wastes have great potential to be used as a protein supplement of low-cost in poultry feeds. The objective was to evaluate the effect of dried salmon silage (DSS) on productive performance and gizzard health of broilers. The DSS was obtained from salmon wastes (S) by acid digestion and co-dried with wheat bran (WB) (70S:30WB). Samples of DSS were evaluated for chemical composition, nitrogen-corrected true metabolizable energy (TMEn), and biogenic amines analyzed by HPLC-UV. A total of 240, 1 d-old broilers were allotted in a randomized design with five diets treatments (0, 4, 8, 12 and 15% of DSS) with 4 replicates and 12 birds per replicate. Diets of starter (0-22 d) and finisher (23-42 d) were prepared. Productive parameters were evaluated. At 22 and 42 days, 12 chicks per treatment were necropsied for examination and determination of gizzard lesions by conventional histopathology. The lesions were classified on a scale of 0 (normal gizzard) to 4 (ulceration of the gizzard) scores. The chemical composition of DSS was: moisture (14.1 \pm 1.0%), crude protein (50.1 \pm 0.8%), ether extract $(5.7 \pm 2.6\%)$, crude fiber $(3.8 \pm 0.5\%)$, ash $(10.7 \pm 0.5\%)$, phosphorus (1.98%) and calcium (1.01%). The TMEn for broiler was 2,612.5 Kcal/Kg. The diets were isoproteic and isoenergetic. There was no significant effect of the percentage of DSS inclusion on percentage of mortality, body weight, daily weight gain, feed intake and feed conversion (p>0.05). The biogenic amines content (mg/100g) of DSS were: histamine (1.8 ± 0.5) , putrescine (4.8 ± 1.7) , cadaverine (8.1 ± 3.6) , and tyramine (12.1 ± 1.9) . Broiler fed diets containing DSS until 15% did not have any detectable signs of macroscopic or microscopic gizzard erosion lesions caused by the DSS inclusion in the diets. The DSS showed high crude protein content. The biogenic amines were low and comparable with fish meal of high quality. The inclusion of DSS until 15% in broiler diets did not have significant effects on productive performance and gizzard health, becoming a lower-cost protein source.

Key words: poultry feeds, salmon wastes, dried-silage

INTRODUCTION

The salmonid farming industry plays an important economic and social role in Chile, being the main salmonproducing country in the southern hemisphere and the second largest farmed salmon producer in the world after Norway. The three main salmonid cultivated species are Atlantic salmon (Salmo salar), coho salmon (Oncorhynchus kisutch) and rainbow trout (Oncorhynchus mykiss), farms being distributed from Puerto Montt to the Aysen coast (SERNAPESCA, 2012). Farming of salmonids is very intensive in Chile, generating a series of potential ecosystem disruptions (Buschmann et al., 2006a) as environmental pollution (Naiman et al., 2002), chemical and drug contaminants (Cabello, 2004), precipitation of organic and inorganic matter in sediment (Tett, 2008), and high mortality rates (Buschmann et al., 1996). Although environmental and sanitary regulations for salmonid aquaculture exist in Chile (Buschmann et al., 2006b) and were improved in late 2007, dead salmon management remains a problem today.

The dead salmon bodies could have a great potential for being used as protein supplement in broiler feeds by ensiling process, which is technologically simpler, feasible and more economical than the manufacture of fish meal (Gildberg, 1993). The acid fish silage is defined as a liquid product produced from the whole fish or parts of it by the action of proteolytic enzymes present in the fish and its combination with acid (e.g. hydrochloric, sulphuric, formic, or citric acid), which accelerate the autolysis and help to break down bone and limit bacterial spoilage (Vidotti et al., 2003). To facilitate transport, storage and the inclusion of this product in animal diets has been studied the co-dried blends of liquefied silage with cereals or legumes meals (Goddard and Perret, 2005). The fish silage co-dried blends from tilapia, sardine and others have been evaluated in broiler diets (Hammoumia et al., 1998, Santana-Delgado et al., 2008). However, there is no information available on dried salmon silage (DSS) on broiler performance. Therefore, the objective of this study was to evaluate the effect of DSS on productive performance and gizzard health of broilers.

MATERIALS AND METHODS

DSS preparation and characterization

The DSS was provided by FIORDOAUSTRAL S.A., Puerto Montt, Chile. The DSS was prepared by thoroughly mixing the whole salmon waste from several species (*Salmo* salar, Oncorhynchus kisutch and Oncorhynchus mykiss) with 85% formic acid (40 mL/Kg) (Oxiquim S.A, Chile), and stored outside in stainless steel tanks at environmental temperatures (range 5-15°C) during 1 to 2 weeks. The DSS was obtained by co-dried of liquid silage with wheat bran (70 parts silage: 30 parts wheat bran) at 100 to 120°C. Ethoxyquin (200 ppm) was added to reduce lipid oxidation.

The chemical and nutritional properties of DSS were performed in duplicate from 20 representative samples. The chemical composition of DSS was conducted by the AgriServices Laboratory, University of Georgia, according to the methodology proposed by the AOAC (1996) for content of moisture, crude protein (N x 6.25), crude fiber, ether extract, ash, phosphorus and calcium. The TME_n was determined by Sibbald method (1976) as modified by Dale and Fuller (1984) using adult Single Comb White Leghorn roosters. The biogenic amines such as histamine, putrescine, cadaverine, and tyramine were determined by high-resolution liquid chromatography UV detector (HPLC-UV) in the laboratory of Bioquality SA., Chile.

Birds, experimental diets and procedures

A total of 240, 1 d-old broilers (Ross 308) were allotted in a randomized design with five diets treatments (0, 4, 8, 12 and 15% of DSS) (Table 1) with 4 replicates and 12 birds per replicate. Diets of starter (0-22 d) and finisher (23-42 d) were prepared (Table 1) and analyzed according to AOAC (1996) for content of moisture, crude protein (N x 6.25), crude fiber, ether extract and ash. Experimental diets and water were provided ad libitum. Body weight (BW), daily weight gain (DWG), feed intake (FI) and feed conversion (FI:BW) were determined. Mortality was recorded daily. At 22 and 42 days, 12 chicks per treatment were necropsied for examination and determination of gizzard lesions by conventional histopathology. The gizzards were fixed in a 10% formalin solution. After dehydration in a Histokinette 2000, Reichert-Jung, tissues were paraffin embedded, cut and stained with hematoxylin-eosin. The lesions were classified on a scale of 0 (normal gizzard) to 4 (ulceration of the gizzard) scores.

Statistical analysis

Results were presented as mean \pm SD. Productive values were compared using ANOVA, and post hoc Tukey's multiple range tests (p < 0.05) were performed through Statgraphics Plus 5 (Statistical Graphics Corp, Rockville, MA, USA).

RESULTS AND DISCUSSION

Characterization of DSS

The chemical composition of DSS (% DM) was as follows: dry matter ($87.7 \pm 0.8\%$) crude protein ($50.1 \pm 0.8\%$), ether extract $(5.7 \pm 2.6\%)$, crude fiber $(3.8 \pm 0.4\%)$, ash $(10.7 \pm$ (0.5%), phosphorus (1.98%) and calcium (1.01%). The moisture content of the DSS was lower than other co-dried fish silages (\approx 33 to 40% DM) (Fagbenro and Jauncey, 1994; Goddard and Perret, 2005). This aspect is positive as allows a safe storage and extended it useful life. The crude protein content was high and it is found in the range reported for several fish silage acids (37 to 58% DM) (Goddard and Perret, 2005; Gerón et al., 2007). The ether extract and ash fractions were lower than other acids silages from tilapia (ash, 23.5% and ether extract, 35.4%) (Gerón et al., 2007) and mixed fishes (ash, 16.9% and ether extract, 21.1%) (Llanes et al., 2011), because DSS was blended with wheat bran a rate of 70:30, respectively, generating a reduction in ether extracts and ash as reported Fagbenro and Jauncey (1994) for co-dried blends of fish silage:

Table 1	1.	Ingredients	of	experimental diets	ŝ.
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Ingredients (%)	Diets of starter (0-22 d)						Diets of finisher (23-42 d)			
	0%	4%	8%	12%	15%	0%	4%	8%	12%	15%
Corn	68.31	68.22	68.13	68.06	67.97	69.41	69.4	69.43	69.46	69.47
Soybean meal	26.02	22.27	18.52	14.77	11.97	11.72	8.93	6.13	3.34	1.02
DSS	0	4	8	12	15	0	4	8	12	15
Gluten meal	-	-	-	-	-	12.86	12.07	11.28	10.49	10.05
Vegetable oil	1	1	1	1	1	1	1	1	1	1
Dicalcium phosphate	1.75	1.60	1.46	1.31	1.2	2.07	1.74	1.41	1.07	0.86
Limenstone	1.94	1.97	2.00	2.02	2.05	1.98	1.93	1.87	1.81	1.79
Salt	0.29	0.25	0.22	0.19	0.18	0.27	0.24	0.21	0.18	0.18
L-Lisine	0.42	0.38	0.34	0.3	0.26	0.42	0.38	0.34	0.30	0.26
DL-metionina	0.12	0.16	0.18	0.2	0.22	0.12	0.16	0.18	0.20	0.22
Premix vitamins ¹	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Premix minerals ²	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CP (%)	23.0	23.6	23.4	23.2	22.9	20.8	20.9	21.0	20.9	20.6

¹Vitamins per kg: A: 10,000 U.I; D3: 3,500 U.I; E: 50 U.I; K3: 2 mg; B1: 2 mg; B2: 8 mg; B6: 4 mg; B12: 0.015 ng; B3: 40 mg; B5: 15 mg; B8: 0.13 mg; B9: 1.5 mg; choline chloride: 400 mg. ²Minerals per kg: Cu: 8 mg; Zn: 80 mg; Fe: 80 mg; Mn: 100 mg; I: 1 mg; Se: 0.25 mg. CP is crude protein.

g/bird), and fee	ed conversion (FG	C, FI:BW).			
Parameters			DSS (%)		
	0%	4%	8%	12%	15%
M (%)	1.7	0	3.3	3.3	3.3
BW	$2,026 \pm 271^{a}$	$2,075 \pm 260^{a}$	$2,133 \pm 224^{a}$	$2,137 \pm 297^{a}$	$2,106 \pm 240^{a}$
DWG	47 ± 6^{a}	48 ± 5^{a}	49 ± 5^{a}	49 ± 7^{a}	49 ± 5^{a}
FI	$6,304 \pm 41^{a}$	$6,549 \pm 30^{b}$	$6,649 \pm 12^{b}$	$6,724 \pm 34^{\circ}$	$6,749 \pm 35^{\circ}$
FC	3.1 ± 0.1^{a}	3.2 ± 0.2^{a}	3.1 ± 0.1^{a}	3.2 ± 0.1^{a}	3.2 ± 0.1^{a}

Table 2. Effect of dried salmon silage (DSS) at different levels on growth performance of broiler: mortality (M%), body weight (BW, g/bird), daily weight gain (DWG, g/bird/day), feed intake (FI, g/bird), and feed conversion (FC, FI:BW).

Values are expressed as mean \pm SD. Means with different superscript letters are different statistically (p < 0.05).

soya meal. The phosphorus and calcium values were high due to the presence of spines and bones in the final product.

The TME_n value of DSS was 2,612.5 Kcal/Kg similar to soybean meal value (2,585 Kcal/Kg) (NRC, 1994), and higher than other protein concentrates commonly used for poultry feed such as cottonseed meal (2,135 Kcal/Kg), canola meal (2,070 Kcal/Kg), sunflower meal (2,060 Kcal/Kg), and meat meal rendered with bone (2,150 Kcal/Kg) (NRC, 1994).

Productive performance and gizzard health of broiler

The diets were isoproteic (Table 1) and isoenergetic. The protein concentration of DSS allowed its inclusion in broiler diets as a replacement for soybean meal and gluten meal. The productive parameters of broiler are showed in Table 2. The mortality percentage obtained at the end of the study was low and similar to that found in commercial farms. No significant differences were observed for BW, DWG and FC between the different feeding groups. FI increased slightly but significantly as the inclusion percentage of DSS was increased. These results are similar to the reported by Johnson et al. (1985) and Santana-Delgado et al. (2008), who did not reported changes on production parameters using similar concentrations of fish silages in broiler diets.

The biogenic amines content (mg/100 g) of DSS were: histamine (1.8 ± 0.5), putrescine (4.8 ± 1.7), cadaverine (8.1 ± 3.6), and tyramine (12.1 ± 1.9). The biogenic amines were low and comparable with fish meal of high quality (Shalaby, 1996). The gizzard lesions of all animals were classified between 0-2 score and could not be established a clear association between degenerative changes of the gizzards mucosa and the concentration of DSS in the diets. In many areas of keratoide degeneration, bacteria and/or yeast were found, which partly explain the findings.

IMPLICATIONS

The conversion of salmon wastes into a new feed ingredient for poultry can contribute to overcoming protein shortages in areas where soybean production is lacking, or could be used to replace partiality the protein concentrates used in the broiler diets.

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Capsaicin influences rumen microbial fermentation and gas production in vitro¹

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ABSTRACT: This study was preliminary to a research project that evaluated the potential for capsaicin to decrease inflammation in cattle. The objective of this research was to evaluate the effects of capsaicin on rumen microbial fermentation and gas production in an in vitro system. Rumen fluid was collected from 2 ruminally-cannulated heifers fed an alfalfa hay-based diet. Strained rumen fluid (50 mL) was mixed with McDougal's buffer (50 mL) and anaerobically incubated at 39°C in 250 mL Erlenmeyer flasks that contained treatments. Treatments were 1 g of ground alfalfa hay that contained either 0% (CON) or 2% (CAP) jalapeño powder (contained 1,280 ppm capsaicin). Gas production was measured from 24 flasks (12 replicates per treatment) that were incubated for 24 h, and gas measurements were recorded at 0, 2, 4, 6, 8, 10, 12, 18, and 24 h. Rumen microbial fermentation products (pH, NH3, and VFA) were collected from 2 runs of 32 incubating flasks that were stopped after 0, 6, 12, or 24 h of incubation (8 replicates per treatment at each incubation time). Data were analyzed statistically as repeated measures using mixed models. A treatment × hour interaction (P = 0.02) occurred for gas production; gas production was not different at 0, 2, 8, 10, 12, 18, and 24 h, but was greater (P < 0.05) for CAP than CON at 4 and 6 h. No treatment × hour interactions ($P \ge 0.24$) occurred for pH, total VFA, and individual VFA proportions. Ammonia concentrations tended to be lower for CAP than CON at 24 h (treatment \times hour, P = 0.12). Ammonia and total VFA concentrations were not different ($P \ge 0.23$) between CON and CAP, and pH tended to be greater (P = 0.11) for CAP than CON. Molar percentages of acetate were greater (P < 0.01), and molar percentages of propionate, butyrate, and valerate were lower (P < 0.01) for CAP than CON. Thus, acetate:propionate ratio was greater (P < 0.01) for CAP than CON. Ruminal pH decreased (P < 0.01), and concentrations of NH3 and total VFA increased (P <0.01) as incubation time increased. These results demonstrate that the addition of 2% jalapeño powder to a ground alfalfa hay substrate altered rumen microbial fermentation and gas production. These effects on rumen microbial fermentation were in favor of acetate production.

Key words: capsaicin, in vitro, rumen, volatile fatty acid

INTRODUCTION

Capsaicin is the major capsaicinoid in hot peppers and is responsible for a pungent sensation when consumed. In addition to its use in cuisine, capsaicin has pharmacological uses because of its role in pain management, cardiovascular, respiratory and other biological systems (O'Neill et al., 2012). Dogan et al. (2004) reported that capsaicin reduced lipopolysaccharide-induced fever in rats, and Demirbilek et al. (2004) demonstrated that capsaicin decreased proinflammatory cytokines and increased anti-inflammatory cytokines in septic rats. Therefore, feeding capsaicin in hot peppers to ruminant livestock may be effective at reducing fever when they have been exposed to stress and disease.

Natural plant extracts, including capsaicin, have been shown to alter rumen microbial fermentation (Cardozo et al., 2004; 2005; 2006). According to Cardozo et al. (2005), the effects of natural plant extracts on rumen microbial fermentation is variable, and is dependent on the ruminal pH. Also, Cazac et al. (2005) demonstrated that ruminal degradation of chile peppers is greater in forage-based diets than in concentrate-based diets. Therefore, the effects of capsaicin on rumen microbial fermentation should be researched further before being evaluated as an antiinflammatory supplement for ruminant animals. The objective of this study was to evaluate rumen microbial fermentation and gas production in response to the addition of jalapeño powder to a ground alfalfa hay substrate in an in vitro system.

MATERIALS AND METHODS

In Vitro Procedure

This research was preliminary to another project that evaluated the potential for capsaicin to decrease inflammation in cattle (Samuelson et al., 2014). All procedures were approved by the Institutional Animal Care and Use Committee of New Mexico State University. Rumen fluid was collected from 2 ruminally-cannulated heifers receiving an alfalfa hay diet. Equal amounts of rumen fluid from each heifer were mixed together and strained through cheesecloth into a prewarmed ($\pm 39^{\circ}$ C) thermos for transportation to the laboratory. The strained rumen fluid was mixed with an equal volume of

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pre-warmed (±39°C) McDougal's buffer (Tilley and Terry, 1963), and 100 mL of this mixture was added to 250 mL Erlenmeyer flasks that contained treatments. The Erlenmeyer flasks were then flushed with CO2 to displace O2 from the gaseous head space, and were fitted with rubber stoppers connected to 250-mL inverted burette cylinders with silicone tubing. Erlenmeyer flasks were incubated at 39°C in a LAB-LINE Orbit Environmental Shaker that provided continuous rotational movement.

Treatments

Treatments were alfalfa hay (ground to pass a 2 mm screen) that contained either 0% (**CON**) or 2% (**CAP**) jalapeño powder (as fed basis); the jalapeño powder contained 1,280 ppm capsaicin. One gram of either CON or CAP was weighed into 250-mL Erlenmeyer flasks before anaerobic incubation with equal volumes of rumen fluid and McDougal's buffer in a LAB-LINE Orbit Environmental Shaker.

Gas Production

For the measurements of gas production, 24 Erlenmeyer flasks containing CON or CAP treatment, rumen fluid and McDougal's buffer were incubated for 24 h in a completely randomized design. All flasks (12 flasks per treatment) were incubated for 24 h without interruption, and the volume of water displacement was recorded as a measure for gas production at 0, 2, 4, 6, 8, 10, 12, 18, and 24 h of incubation. Displacement of water by the production of gas was measured using 250-mL burette cylinders that were inverted into 600 mL beakers filled with water. The spout of the burette cylinders were connected to the incubating Erlenmeyer flasks with a silicone tube to capture the production of gas.

Rumen Microbial Fermentation

For the measurements of rumen microbial fermentation products, 2 runs of 32 Erlenmeyer flasks containing CON or CAP treatments, rumen fluid and McDougal's buffer were incubated for 0, 6, 12, or 24 h in a randomized complete block design. This experiment was blocked by run because of limited space in the incubator. For both runs, there were a total of 8 replicate flasks per treatment at each incubation time. Erlenmeyer flasks that were assigned to an incubation hour were removed from the incubator when the appropriate time had elapsed (other fermentation flasks were not disturbed during this process). The pH of the fluid was measured immediately after the termination of fermentation using a portable pH meter (Mettler Toledo 8603, Schwerzenbach, Switzerland). Then, two 10-mL samples of fluid were transferred from each incubation flask to separate scintillation vials and immediately frozen at -20°C to stop further fermentation by anaerobic micro-organisms. Samples were later thawed, centrifuged (Eppendorf, Hamburg, Germany) at $28,000 \times g$ for 20 min, and the supernatant was analyzed for VFA and NH3 concentrations. Individual VFA concentration were determined using capillary gas chromatography (Varian 3400, Varian Inc., Walnut Creek, CA) in accordance to May and Galyean (1996), and NH3 concentrations were determined according to the procedure of Broderick and Kang (1980) adjusted for a micro-plate reader (ELX 808 Ultra Micro Reader, Bio-Tek Instruments Inc., Winooski, VT).

Statistical Analysis

All data were analyzed statistically as repeated measures using mixed models (SAS Inst. Inc., Cary, NC). Flask was the experimental unit. For gas production, the experiment was a completely randomized design. The statistical model included treatment, hour, and treatment × hour interaction. The covariance structure was compound symmetry. For rumen microbial fermentation products and pH, the experiment was a randomized complete block design. The statistical model included treatment, hour, and treatment × hour interaction. Block was random, and the covariance structure was autoregressive order one. This experiment was blocked by run (collection date) because space in the incubator limited the number of flasks that could be incubated at the same time. Differences were considered significant when P < 0.05.

RESULTS

An interaction between treatment and hour (P = 0.02) was observed for in vitro gas production; gas production was not different between CON and CAP at 0, 2, 8, 10, 12, 18, and 24 h, but was greater (P < 0.05) for CAP than CON at 4 and 6 h of incubation (Figure 1). No interactions between treatment and hour ($P \ge 0.24$) were observed for pH, total VFA concentrations, and molar percentages of individual VFA (Figure 2). Ammonia concentrations tended to be lower for CAP than CON at 24 h (treatment × hour, P = 0.12). Ammonia and total VFA concentrations were not different ($P \ge 0.23$) between CON and CAP, and pH tended to be greater (P = 0.11) for CAP than CON. Rumen fluid from in vitro fermentations containing CAP had greater (P



Figure 1. In vitro gas production from equal volumes of rumen fluid and McDougal's buffer when incubated with 1 g ground alfalfa hay that contained either 0% (**CON**) or 2% (**CAP**) jalapeño powder (as fed basis); the jalapeño powder contained 1,280 ppm capsaicin.



Figure 2. In vitro pH, NH3, and VFA concentrations of equal volumes of rumen fluid and McDougal's buffer when incubated for 0, 6, 12, and 24 h with 1 g ground alfalfa hay that contained either 0% (**CON**) or 2% (**CAP**) jalapeño powder (as fed basis); the jalapeño powder contained 1,280 ppm capsaicin.

< 0.01) molar percentages of acetate, and lower (P < 0.01) molar percentages of propionate, butyrate, and valerate, than in vitro fermentations containing CON. Therefore, the acetate:propionate ratio was greater (P < 0.01) for fermentation flasks containing CAP compared with CON. Regardless of treatment, pH decreased (P < 0.01), and concentrations of NH3 and total VFA increased (P < 0.01) as incubation time increased.

DISCUSSION

No significant differences between CAP and CON for ruminal pH, NH3, and total VFA concentrations suggest that CAP had little or no effects on rumen microbial fermentation in vitro. However, small increases in gas production as well as greater molar percentages of acetate, and lower molar percentages of propionate, butyrate, and valerate indicated that CAP shifted rumen microbial fermentation in favor of acetate production. This increase in the ratio of acetate:propionate may have resulted in the tendency for pH to increase in response to CAP. The observed shifts in individual VFA proportions are consistent with the results of Cardozo et al. (2005), who reported that Capsicum annuum containing 12% capsaicin increased acetate:propionate ratios when the in vitro ruminal pH was 7.0. Cardozo et al. (2005) also reported that capsaicin decreased both total VFA concentrations and NH3 concentrations. Although, total VFA concentrations were not affected in this study, CAP tended to decrease NH3 concentrations, which is an indication that amino acid deamination was perhaps decreased by capsaicin (Cardozo et al., 2005).

In conclusion, the results of this study indicated that the addition of 2% jalapeño powder (contained 1,280 ppm capsaicin) to a ground alfalfa hay substrate alters rumen microbial fermentation and gas production in an in vitro batch culture system. These effects on rumen microbial fermentation were in favor of acetate production. Further research is necessary to evaluate the degradation of capsaicin by rumen microbes so that the post-ruminal supply of capsaicin as a potential anti-inflammatory for ruminant animals can be quantified.

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Levels of supplementation in the performance for beef heifers, in grazing, during the dry season

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ABSTRACT: Productive performance of crossbreds Nellore beef heifers, in the growing stage receiving different supplement levels for three months grazing Brachiaria decumbens pasture were evaluated. The area was divided in five paddocks of 2.5 ha, with dry matter availability and potentially of dry matter digestible of 3.85 and 2.12 t/ha, respectively. Forty beef heifers of 191 ± 3.99 kg initial weight and 8.5 ± 0.15 months of age were assigned in a completely randomized experimental design with five treatments, and four supplementation levels. Mineral mixture (MM) (60 g/day) and multiple supplements, formulated to supply different supplementation levels in the amounts of 0.5, 1.0, 1.5 and 2.0 kg/animal more MM (60 g/day) were evaluated. The supplement had 300 g of crude protein/kg. Animals not responded significantly (P > 0.05) to the use of multiples supplements to final body weight. However, the heifers than received supplement won more weight (P £ 0.01) (372 g vs. 92g of average daily gain), in addition to highers ($P \pm 0.01$) serum N-urea levels (20.14 vs. 12.09 mg/dL), when compared with heifers fed diets supplied only with mineral mixture. In conclusion, increasing the amount of protein supplement during the dry season, increased of performance of beef heifers. However, despite increase productive performance and intake of energy during dry season, additional higher levels of supplementation resulted in lower increment in growth.

Key words: beef cattle, daily gain, pasture, protein, supplements

INTRODUCTION

In the dry season of years, due to unfavorable climatic conditions, occurs reduction of forage quality on the content of crude protein and high concentrations of neutral detergent fiber with large lignified portion. This way have been used in an increasing scale by producers of beef cattle, mineral salt with urea and supplements protein low consumption, especially for animals in the growing phase, in order to meet the nutritional deficiencies of pasture. However, the weight gains obtained are still negative or very low and may compromise the productive system, since replacement heifers or killing staying at the property in the growing phase, for a long period. In this context, the use of larger amounts of energy as food supplements could bring benefits to the production system, increasing the weight of the heifers, conception rate and decreasing the time in the growing phase.

This study aimed to evaluate the performance in beef heifers, in growing phase, receiving different offers multiple supplements, in pasture *Brachiaria decumbes*, Stapf, during dry season.

MATERIALS E METHODS

The experiment was conducted in the Cattle Sector -Universidade Federal de Viçosa, located in Viçosa – MG - Brazil, during dry season, between the months of July to September 2006, 84 days of works. An experimental area comprised of five paddocks of 2.5-ha each was used, formed by Brachiaria decumbens, each batch allocated in a paddock. Change of the animals was carried out between the paddocks every 14 days.

Experimental Design and Treatments

Forty heifers crossbred were used, ³/₄ Nellore ¹/₄ Holstein, with age and average weight of 8.5 ± 0.15 months and 191.0 \pm 3.99 kg, respectively. Heifers were randomly allotted to five treatments with eight replications, which were evaluated in four different supplements offers and mineral mix. The supplements were: mineral mixture (MM), control group, and supplements composed of the following ingredients: soybean meal, ground corn grain, mixture of urea more ammonium sulfate (9:1), MM was supplied in the amount of 60 g/animal, regardless of the treatment it was in, being the amounts of supplements offered in 0.5, 1.0, 1.5 e 2.0 kg/animal. The supplements attended 13, 26, 39 e 52% the requirements for total digestible nutrients, respectively (NRC, 1996), for a heifers of 190 kg and average daily gain of 0.4/kg, satisfying 25, 50, 75 e 100% of the requirements of CP, respectively. The chemical composition of supplements and pasture can be found in Table 1.

The heifers were weighed without fasting at beginning of study and every 28 days, always in the morning, without allowing water intake before weighing. On the 14th day of the trial period, pasture samples were taken by cutting a 5 cm soil, of four areas of 0.25 m^2 , randomly, within each paddock to assess the total available dry matter e potentially digestible dry matter (**PDDM**)/ha.

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		Leve	l of sup	plemen	ıt (kg)	
Items	MM	0.5	1.0	1.5	2.0	Pasture 4
			Proport	tion (%)	
Mineral mixture ¹	100.0					
Urea/sulfate de ammonium (9:1)	-		2.	50		
Soybean meal	-		40	.00		
Ground corn grain	-		57	.50		
Total	100.0		100	0.00		
Chemical composition						
Dry matter (DM)	98.20					$35.84 \pm 0,37$
Crude protein (CP) ²			31	.73		7.38 ± 0.74
Neutral detergent fiber (NDF) ²			8.	94		62.79 ± 2.56
Etheric extract (EE) ²			1.	80		1.37 ± 0.24
Organic matter (OM) ²			94	.21		90.80 ±0.26
Non Fibrous carbohydrates (NFC) ^{2,3}			58	.23		19.29 ±2.77
Neutral detergent fiber indigestible (NDFi) ²			2.	73		28.39 ±2.33

 Table 1. Perceptual composition the of supplements, with based on natural matter and chemical composition of pasture and supplement

¹Perceptual composition: phosphate bicalcium, 50.00; sodium chloride, 47.15; zinc sulphate, 1.50; copper sulphate, 0.75, cobalt sulphate, 0.05; potassium iodate, 0.05 and magnesium sulphate 0.5%; ²% in DM; ³NFC = 100 - [(%CP - %CPurea + %urea) + %NDFashcp + %EE+ % ashes] in supplements; ⁴ means and standard-error of the means the of samples of simulated grazing obtained during the experiment.

Evaluate the chemical composition of the forage intake, also on the 14th day of each trial period collecting the pasture by grazing simulation manual.

Laboratorial Analysis

Proceeded to analyses the content of PDDM the total mass of the pasture seconds Paulino et al. (2006), by the following equation: PDDM = 0.98 (100 - NDF) + (NDF - NDFi) where, NDF = neutral detergent fiber and NDFi = neutral detergent fiber indigestible. The samples were dried in a stove of forced ventilation at 60° C for 72 hours, and ground to pass through a 1.0 mm screen. The simulated grazing na supplements were analyzed for dry matter (**DM**), crude protein (**CP**), etheric extract (**EE**), neutral detergent fiber (**NDFi**), ashes e neutral detergent fiber indigestible (**NDFi**), obtained in situ by incubation for 264 hours.

Statistical Analysis

This study was carried on using a completely randomized design, and comparisons between the supplements levels performed by orthogonal polynomial contrasts: control (MM vs. supplements), linear and quadratic, and adjustments of regression equations; being P-values \pm 0.05 were considered different.

RESULTS AND DISCUSSION

Characteristics of Pasture

Availabilities of total dry matter (**TDM**) and potentially digestible dry matter (**PDDM**) were the first to the second

period increased, already in the third period occurred reduced availability of forage due to unfavorable climatic conditions. The average availability of TDM and PDDM were of 3.85 and 2.12 ton/ha, with a potential digestibility of forage available from 55.06%, most of this is likely to be used when providing supplements to animals (Paulino et al., 2006); mainly in the dry season where the average content of CP of the pasture was 7.38%. The offer was 8.39 and 4.63 kg of TDM and PDDM/100 kg of body weight/day, respectively, with a PDDM within recommended by Paulino et al. (2004), values 4.0 to 5.0 kg of PDDM/100 kg of body weight/day, to support Cattle Precision which entails the exploration of the genetic limits of animals created on pasture.

Productive Performance

Due to the qualitative characteristics (Table 1) and quantitative presented by pasture, heifers responded to the use of multiple supplements ($P \pm 0.01$) gaining more weight (281 g/day) compared to heifers receiving only MM (373 vs. 92 g/day, respectively).

The final body weight of heifers showed no significant difference (P > 0.05). However, the final body weight of the supplemented animals was higher in 18.67 kg, when compared with the control group (MM), which can accelerate return on total capital invested, due to the short time that the animals will be in the rearing in growing phase (Table 2).

Supplemented heifers had higher final height ($P \pm 0.05$), which confirms the benefits of supplementation on performance when compared to animals receiving MM (129.1 vs. 124.4 cm, respectively).

V		Leve	el of sup	plementa	ation		Contras	st	CV (%)
variable	MM	0.5	1.0	1.5	2.0	C ¹	L^1	Q^1	
IBW^2	195.4	194.0	186.0	185.2	196.8	ns	ns	ns	-
FBW^2	203.1	221.9	211.6	218.9	234.7	ns	ns	ns	14.60
IH^2	119.9	119.2	120.2	119.7	119.7	ns	ns	ns	-
FH ^{1,2}	124.4	129.0	130.1	128.2	129.1	**	ns	ns	3.53
WHRI ²	1.63	1.62	1.54	1.54	1.64	ns	ns	ns	-
WHRF ²	1.63	1.71	1.62	1.70	1.81	ns	ns	ns	12.11
ADG ^{1,2}	92	332	305	402	452	*	ns	ns	28.33
Isupp ⁴	0.06	0.50	1.00	1.27	1.67	-	-	-	-
SNU ^{1,3}	12.09	17.30	20.88	21.77	20.62	*	**	ns	18.66

Table 2 – Minimums squares means and coefficient of variation (CV, %) to means biometric, levels of serum N-urea (SNU, mg/dL), intake; in according with the levels of supplementation

¹ C = control, L = linear and Q = quadratic, significant the 1% (*) and 5% (**) of probability and ns = no significant; ² IBW = initial body weight, kg; FBW = final body weight, kg; IH = initial height, cm; AF = final height, cm; WHRI and WHRF = weight and height ratio initial and final, respectively, ADG = average daily gain, g/animal; ³ SNU = 17.436 + 2.165SL (r² = 89,23), where SL = supplementation level; ⁴ Isupp = intake of supplement, with based on natural matter (kg/animal/day).

The average daily gain (**ADG**) of the animals showed increasing linear responses to increasing levels of supplements ($P \pm 0.01$). The lowest level of supplementation was able to promote a ADG of 332 g, being three times larger than the gain observed for the control group, which demonstrates the benefits of supplementation, during dry season of year, even when the forage has a crude protein content 7%, enough to maintain rumen activity. Similar results were observed for Porto et al. (2011), supplementing young bulls with different offers on work done in parallel to this study.

Lazzarini et al. (2013) supplemented with both starch and nitrogenous compounds to cattle grazing on low-quality tropical forage is characterized by an interactive effect that increases nitrogen retention by the animals. This interactions can explain the increasing in ADG even at low levels of supplementation.

IMPLICATIONS

The supply of supplements in increasing levels during dry season, results in increasing the productive performance of the heifers.

Increasing supplements increased energy intake during the dry season, may reduce the growing phase, due to the increased performance of heifers; and can result in more than acceptable conception rates and slaughter weight of heifers. However, the extent of this performance is reduced as the supply of supplement increases.

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Plasma amino acids of wether lambs supplemented with novel feed products to reduce locoweed toxicity¹

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ABSTRACT: Locoweeds impair performance and may cause death in grazing livestock. Novel feed products are needed that counter or minimize the toxic effects of locoweed. The objective was to evaluate effects of 3 feed product formulations on plasma AA of lambs consuming locoweed. Forty wether lambs $(39 \pm 0.4 \text{ kg BW})$ were housed individually and fed 620 g/d of alfalfa hay and 100 g/d of cornbased feed twice daily in equal portions for 20 d. Lambs were equally divided into 4 blocks, and randomly assigned to 1 of 5 treatments within each block. Treatments were: no locoweed or feed products (CON); 20 g/d of locoweed (LOCO); 20 g/d of locoweed and 50 g/d of feed product 1 (AK1); 20 g/d of locoweed and 50 g/d of feed product 2 (AK2); 20 g/d of locoweed and 50 g/d of feed product 3 (AK3). Locoweed and feed products replaced alfalfa hay in the basal diet. Plasma from jugular venous blood was collected on d 0, 3, 6, 9, 12, 15, 18, and 20 of treatment. Treatment \times day interactions (P < 0.05) were detected for plasma Gly and Thr, but not for other AA (P > 0.05). Plasma Gly was not different among treatments on d 0, but was greater for LOCO, AK1, AK2 and AK3 than CON on d 3 (except AK1 and AK2), 6, 9, 12, 15, 18, and 20. Plasma Thr was not different among treatments on d 0 and 6, but was greater for LOCO, AK1, AK2 and AK3 than CON on d 3 (except AK1 and AK2), 9 (except AK2), 12 (only AK2), 15, 18, and 20. Plasma, Leu, Met, Val, Ala, Asn, and Pro were greater (P < 0.05), while Glu was lower (P < 0.05) 0.05) in lambs fed treatments containing locoweed compared with CON. The increase in plasma AA in lambs fed locoweed suggests that AA uptake was impaired and(or) tissue protein degradation was increased. Plasma concentrations of His, Ile, Lys, Phe, Trp, Asp, Gln, Ser, and Tyr were not different (P ≥ 0.07) between lambs fed treatments containing locoweed and CON. Lambs supplemented with AK1, AK2, or AK3 had plasma AA that were not different ($P \ge 0.07$) than lambs fed LOCO. We conclude that locoweed consumption alters plasma AA in lambs and that addition of novel formulations did not counter the effects of locoweed on plasma AA.

Key words: amino acids, locoweed, sheep, swainsonine

INTRODUCTION

Locoweeds are poisonous legumes that are responsible for substantial economic losses in livestock (Nielsen and James, 1992). Swainsonine, a fungal endophyte alkaloid (Cook et al., 2011), is the primary toxicant in locoweed (Molyneux and James, 1982). Swainsonine alters glycoprotein processing, which results in a number of subclinical and clinical disorders in livestock consuming locoweed. Many proteins that are essential for AA metabolism are glycosylated, such as rBAT and 4F2 (Mailliard et al., 1995). Swainsonineinduced alteration of essential glycoproteins could impair AA metabolism in livestock consuming locoweed (Pan et al., 1993).

Novel feed products are needed that counter or minimize the toxic effects of locoweed. For example, a bacterial strain (YLZZ-1) isolated from the soil surrounding *Oxytropis kansuensis* was found to degrade swainsonine in culture (Zhao et al., 2009). Furthermore, a novel feed supplement containing a combination of bacterial cell walls, yeast, and enzymes decreased subclinical symptoms of locoweed toxicity in sheep (personal communication, A. Temple, 2010).

We hypothesized that consumption of locoweed will alter AA metabolism in lambs and such alterations can be countered by feeding novel products. The objective was to evaluate the effects of 3 Agri-King Inc. (Fulton, IL) feed product formulations on plasma AA concentrations of wether lambs consuming locoweed.

MATERIALS AND METHODS

Experimental Design, Animals, and Treatments

Experimental procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. The experimental design was a randomized complete block, with 4 experimental periods (block) and 5 treatments. Forty wether lambs (initial BW = 39.0 ± 0.4 kg) were randomly and equally assigned to 4 experimental periods. Within each experimental period, lambs were randomly assigned to 1 of 5 dietary treatments. Treatments (Table 1) were: no locoweed or feed products (**CON**); 20

¹Authors acknowledge A. Temple and Agri-King, Inc. for supply of feed products and support with sample analysis, and C. Ikard for assistance with AA data entry.

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Table 1. Dietary	treatments fe	d to	lambs
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		Treatments					
Item	CON	LOCO	AK1	AK2	AK3		
Ingredient, g/d							
Alfalfa hay	620	600	550	550	550		
Corn grain	95	95	95	95	95		
Feed product ¹	0	0	50	50	50		
Locoweed ²	0	20	20	20	20		
Molasses	5	5	5	5	5		
Nutrient, % DM							
OM	88.8	88.5	88.5	88.5	88.8		
NDF	48.4	48.7	47.7	49.9	48.1		
ADF	35.8	36.2	35.8	37.6	36.2		
CP	18.3	18.4	17.3	17.5	17.7		
Swainsonine ³							
mg/kg DM	0	148.8	145.1	150.3	122.9		

¹Novel feed products (Agri-King Inc., Fulton, IL) containing rice hulls (carrier) and a combination of bacterial cell walls, yeast, and enzymes.

²Astragalus allochrous (half moon locoweed).

³Analyzed using the modified α -mannosidase inhibition assay as described by Taylor and Strickland (2002).

g/d of locoweed (LOCO); 20 g/d of locoweed and 50 g/d of feed product 1 (AK1); 20 g/d of locoweed and 50 g/d of feed product 2 (AK2); 20 g/d of locoweed and 50 g/d of feed product 3 (AK3). Treatments were mixed with a basal diet (620 g/d of alfalfa hay and 100 g/d of corn-based feed); locoweed and feed products replaced alfalfa hay in the basal diet. Mixed treatment diets were fed individually in equal portions twice daily (0730 and 1930) for 20 d. Lambs were housed in individual feeding pens from d 1 to 14 for adaptation to dietary treatments and in metabolism crates from d 15 to 20 for urine and fecal collections (Allataifeh et al., 2012a).

Locoweed (*Astragalus allochrous*) was collected in April in southeast New Mexico, allowed to air dry, and passed through a forage chopper (The Western Bear Cat No 5A, Western Land Roller Co., Hastings, NE) to reduce particle size. The locoweed contained approximately 0.47% swainsonine (also verified by the USDA-ARS Poisonous Plant Research Laboratory, Logan, UT), which resulted in a daily swainsonine dose of 2 mg·kg BW-1 for lambs fed locoweed.

Sample Collections and Analysis

Jugular vein blood samples were collected (10-mL Monoject sodium heparin tubes) at 4 h after the morning feeding on d 0, 3, 6, 9, 12, 15, 18, and 20. Samples were centrifuged (1,500 \times g for 20 min at 5°C; Sorvall RT6000B, Thermo Electron Corp., NC), and plasma was transferred into 7-mL polypropylene vials and stored at -20°C for AA analysis.

Plasma AA concentrations were determined using a commercially available kit (EZ:faast ref. No. KG0-7165, Phenomenex, Torrance, CA) via GLC (CP-3800, Varian, Walnut Creek, CA). This kit supplied the GC column, reagents, preparation vials and racks, and standards. The

mixture of eluted sample was allowed to separate into 2 layers. The upper organic layer was used for AA analysis by GC, with 2 μ L injection volume, 250°C inlet temperature, carried by a constant flow (1.5 mL/min) of helium gas, with 320°C for the detector. This procedure is described by Waggoner et al. (2009).

Statistical Analysis

The experiment was a randomized complete block design, and data were analyzed as repeated measures using mixed models (SAS Inst. Inc., Cary, NC). The experimental unit was lamb and experimental period served as the blocking factor. The statistical model included treatment, day, and treatment × day interaction as fixed effects, and experimental period was the random effect. Compound symmetry covariance structure was specified for repeated measures. When treatment × day interactions were not significant (P > 0.10), single degree of freedom contrasts were used to compare CON with the average for all other treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3. Differences among treatments were considered significant when P < 0.05.

RESULTS

Treatment × day interactions (P < 0.05) were detected for plasma Gly and Thr concentrations (Fig. 1). Plasma Gly concentration was not different among treatments on d 0, but was greater for LOCO, AK1, AK2 and AK3 than CON on d 3 (except AK1 and AK2), 6, 9, 12, 15, 18, and 20. Plasma Thr was not different among treatments on d 0 and 6, but was greater for LOCO, AK1, AK2 and AK3 than CON on d 3 (except AK1 and AK2), 9 (except AK2), 12 (only AK2), 15, 18, and 20. Treatment × day interactions were not detected for other plasma AA (P > 0.05). Plasma essential AA, Leu, Met, Val, and nonessential AA, Ala, Asn, and Pro (Table 2), were greater (P < 0.05), while the concentration of Glu was lower (P < 0.05) in lambs fed locoweed compared with CON. Plasma His, Ile, Lys, Phe, Trp,

Asp, Gln, Ser, and Tyr were not different ($P \ge 0.07$) between lambs fed treatments containing locoweed and CON. Plasma AA in lambs supplemented with AK1, AK2, or AK3 were not different ($P \ge 0.07$) than plasma AA in LOCO lambs.



Figure 1. Plasma Gly and Thr of lambs exposed to locoweed toxicity and supplemented with novel feed products. Treatment \times day interaction (P < 0.05) was detected for Gly and Thr of lambs exposed to locoweed toxicity and supplemented with novel feed products. Treatments were no locoweed or feed products (CON); 20 g/d of locoweed (LOCO); 20 g/d of locoweed and 50 g/d of feed product 1 (AK1; Agri-King Inc. Fulton, IL); 20 g/d of locoweed and 50 g/d of feed product 2 (AK2); 20 g/d of locoweed and 50 g/d of feed product 3 (AK3). Treatments were mixed with a basal diet (620 g/d alfalfa hay and 100 g/d cornbased feed); locoweed and feed products replaced alfalfa hay in the basal diet.

							Contrasts			
			Treatments	s ²			CON vs	LOCO	LOCO	LOCO
Item	CON	LOCO	AK1	AK2	AK3	SEM	Other	vs AK1	vs AK2	vs AK3
Essential AA	. ³ , μ <i>M</i>									
His	45.1	38.3	39.0	42.6	45.1	2.95	0.25	0.87	0.30	0.11
Ile	59.7	68.1	61.1	64.6	67.2	5.14	0.13	0.13	0.44	0.83
Leu	86.2	109	94.5	101	104	9.09	0.01	0.07	0.31	0.54
Lys	38.8	34.3	33.8	34.3	36.2	4.15	0.07	0.86	0.98	0.51
Met	11.9	17.2	15.2	15.4	15.3	1.55	< 0.01	0.09	0.12	0.10
Phe	35.0	38.6	35.6	37.8	41.2	2.92	0.20	0.35	0.78	0.42
Trp	29.4	26.2	23.7	26.8	28.3	4.17	0.16	0.38	0.84	0.46
Val	147	181	157	170	172	17.0	0.02	0.05	0.34	0.45
Nonessential	AA^4 , μM									
Ala	189	231	207	224	219	20.4	0.04	0.19	0.69	0.54
Asp	2.27	1.62	1.57	1.89	2.06	0.43	0.20	0.91	0.55	0.35
Asn	33.6	42.9	38.6	42.7	46.3	6.00	< 0.01	0.27	0.96	0.38
Glu	198	161	148	159	162	16.9	< 0.01	0.45	0.90	0.95
Gln	205	223	206	243	248	20.9	0.19	0.49	0.38	0.29
Pro	101	137	125	132	133	12.9	< 0.01	0.15	0.51	0.62
Ser	163	209	177	206	216	29.7	0.07	0.22	0.90	0.80
Tvr	44.6	49.6	42.5	47.8	49.0	3.57	0.43	0.10	0.68	0.90

Table 2. Plasma AA of lambs exposed to locoweed toxicity and supplemented with novel feed products¹

¹Blood samples were collected from the jugular vein of each animal at 4 h after feeding on d 0, 3, 6, 9, 12, 15, 18, and 20.

²Treatments were no locoweed or feed products (CON); 20 g/d of locoweed (LOCO); 20 g/d of locoweed and 50 g/d of feed product 1

(AK1; Agri-King Inc. Fulton, IL); 20 g/d of locoweed and 50 g/d of feed product 2 (AK2); 20 g/d of locoweed and 50 g/d of feed product 3 (AK3). Treatments were mixed with a basal diet (620 g/d alfalfa hay and 100 g/d corn-based feed); locoweed and feed products replaced alfalfa hay in the basal diet.

³Does not include plasma concentrations of Arg. A significant treatment × day interaction (P < 0.05) for Thr.

⁴Does not include plasma concentrations of Cys. A significant treatment \times day interaction (P < 0.05) for Gly.

DISCUSSION

Effects of Feeding Locoweed

Plasma essential and non-essential AA were found in greater concentrations in lambs fed locoweed compared with CON lambs. Allataifeh et al. (2012a) reported a negative N balance for lambs fed locoweed compared with lambs not fed locoweed. This suggested that swainsonine, from locoweed consumption, may have increased catabolic pathways in AA metabolism. In the current study, the greater plasma AA concentrations observed for all groups fed locoweed versus CON was perhaps because of a decrease in the uptake of plasma AA for protein synthesis. This could result in greater deamination of AA, which is supported by the decreased N retention reported in the study of Allataifeh et al. (2012a).

The negative N balance for locoweed-fed lambs reported by Allataifeh et al. (2012a) could also imply that there was an increase in tissue protein degradation, which would result in an increase in AA into the blood. A tendency for serum urea N to increase and a significant increase in NEFA (Allataifeh et al., 2012b) is a very convincing argument that mobilization of both tissue protein and fat stores occurred in lambs fed locoweed versus CON animals (Allataifeh et al., 2012b).

Effects of Feeding Novel Products

Unpublished results (personal communication, A. Temple, 2010) indicated that novel product formulations containing a combination of bacterial cell walls, yeast, and enzymes seemed to alleviate locoweed toxicity in sheep. However, our data indicated that addition of novel formulations containing various combinations of the same products did not counter effects of locoweed consumption on plasma AA.

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Effects of inorganic trace mineral or amino acid complexed trace mineral supplementation of barley-based receiving and finishing diets on growth performance, carcass merit and health of growing-finishing feedlot steers fed ractopamine¹

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ABSTRACT: Improvements in growth performance, carcass yield and quality and health from feeding amino acid trace mineral (AATM) complexes have been demonstrated for growing-finishing beef cattle fed primarily corn-based diets. Objectives were to evaluate whether similar responses would be observed in growing-finishing feedlot cattle fed barley-based diets and provided ractopamine (RAC) for the final 35 d prior to slaughter. Twenty pens of crossbred steers (n=4,542; initial BW = 270 kg) were fed starting diets providing equal levels of Zn, Mn Cu, and Co from either inorganic sources (CON) or AATM. Finishing diets provided CON steers 360 mg Zn•hd⁻¹•d⁻¹ from either zinc sulfate or AATM. All cattle were fed 200 mg⁻¹•hd⁻¹•d⁻¹ RAC for 35 d prior to slaughter. Live animal performance was not affected (P >0.10) by treatment. Overall carcass weight and total calculated carcass gain was numerically (P = 0.15) greater in CON cattle compared to AATM cattle. The percentage of carcasses grading in the Yield Grade 1 category was increased (P <0.01) in steers fed AATM. The percentage of cattle grading in the Yield Grade 2 and Yield Grade 3 categories were increased ($P \le 0.04$ and $P \le 0.02$, respectively) in CON cattle. Calculated yield grade was improved ($P \le 0.08$) for AATM steers indicating an overall increase in lean meat yield from feeding AATM with RAC. Other observations made in this study suggested AATM may have improved overall health status of cattle as indicated by a decrease ($P \le 0.10$) in overall numbers of animals pulled for medical treatment as well as a decrease ($P \le 0.03$) in the total number of medical treatments administered. Results from this study suggested that feeding AATM in a barley-based feeding program using RAC may produce an improvement in carcass lean meat yield with no decrease in quality grade and may help improve overall health status and response to medical treatment.

Key words: Feedlot cattle; ractopamine; zinc

INTRODUCTION

Development of production technologies over the past 20 years has allowed total U.S. beef production to increase

while overall numbers of cattle have decreased (USDA, 2010). Among these technologies, beta-agonists (BA), such as RAC and zilpaterol (ZIL) have the physiological effect of increasing protein accretion in skeletal muscle and decreasing fat deposition in beef carcasses (Mersman, 1998), thereby increasing carcass weight and lean meat yield. Binding of BA, such as RAC, to a G-protein coupled receptor (GPCR) on the cell membrane increases cyclic adenosine monophosphate (cAMP) concentrations which serve to initiate a series of physiological events which increase lean meat yield in finished beef cattle (Mills, 2002). Because many of these GPCR's require Zn, having an adequate supply of available Zn may be an important factor in allowing the full expression of BA. However, there is little information regarding dietary requirements of Zn or other trace minerals for cattle fed BA. In vitro research has suggested 1uM Zn concentration potentiated the response of RAC by increasing cAMP activity of cultured bovine satellite muscle cells (Harris et. al., 2013). Field research has also shown additive responses to RAC when Zn-AATM complexes are fed to feedlot cattle provided RAC (Zinpro Technical Bulletin, 2007; Zinpro Technical Bulletin, 2012). In these previous research studies, processed corn has been the primary grain source. The objective of this study was to evaluate the relative response of dietary trace mineral source on growth performance, health and carcass characteristics in steers fed RAC in barley-based growing and finishing diets.

MATERIALS AND METHODS

All animals used in the current study were handled humanely as dictated through SOPs designed by the consulting feedlot veterinarians, trial monitors, and investigators. Crossbred steer calves (initial BW. 281 kg) were procured from auction markets and received at the study site in Alberta, Canada during November, 2011. Cattle were allowed to rest for approximately 12 to24 h following arrival and provided grass/legume hay and allowed *ad libitum* access to fresh water prior to processing and allocation to treatment pens.

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Animals were visually appraised upon arrival at the study site and at processing. Animals determined to be in poor health (temperature $\geq 40.6^{\circ}$ C), lame, not exhibiting conformational soundness, in poor body condition, and/or had low BW, were removed from inclusion in the study. Cattle were processed and allocated to replicate groups as they were received into the feedyard. At processing, all animals were identified by a Canadian Cattle Identification Agency (CCIA) tag, which was scanned via computer and tag numbers electronically recorded. Individual BW was recorded and initial pen weight calculated by summing individual weights. Two visual ear tags were also applied during processing, to provide a unique animal identification number and to denote pen assignment for the study. Both tags were cross-referenced with the CCIA number. All cattle were administered a modified live virus vaccine for infectious bovine rhinotracheitis (IBR) virus, bovine virus diarrhea (BVD) virus (Type 2), bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI-3), Mannheimia haemolytica and Pasteurella multocida. Cattle were also vaccinated for various clostridial pathogens and treated with an external endectocide and a metaphylatic dose of long-acting oxytetracycline (Tetradure LA 300[®]; 30 mg oxtetracycline/kg BW; Merial Canada Ltd; Baie-D>Urfe, Quebec). Incoming cattle received an implant in the left ear at initial processing (Ralgro[®], 36 mg zeranol, Merck Animal Health) and were reimplanted at 70 days on feed (DOF) with Revalor S® (120 mg trenbolone acetate, 24 mg estradiol; Merck Animal Health).

Replicates were sequentially completed as cattle were received into the feedlot over a three week period. In total, 4,542 steers were randomized to 20 pens that comprised 10 replicates composed of 2 treatments per replicate. Mean pen stocking density averaged 227 hd• pen⁻¹(range 205 to 275 hd• pen⁻¹) with each pen having approximately 25 to 30.5cm linear bunk space and approximately 11.6 to 14 m² pen space•animal⁻¹. Water tanks allowed for approximately 1.9 cm of linear water unit space per animal. Treatments were randomly assigned to pen, prior to the initiation of the trial, within a predetermined replicate. Replicates were filled within 3 d of initial receiving of cattle at which time dietary treatments were initiated.

Treatments evaluated in the study were:

- Treatment 1 (CON): Diet formulated to provide 360 mg Zn•hd⁻¹•d⁻¹, 200 mg Mn•hd⁻¹•d⁻¹, 125 mg Cu•hd⁻¹•d⁻¹, and 12 mg Co•hd⁻¹•d⁻¹ from inorganic sources, for the first 30 d of the feeding period followed by transition and finishing diets that provided 360 mg Zn•hd⁻¹•d⁻¹from zinc sulfate.
- Treatment 2 (AATM): A diet formulated to provide 360 mg Zn•hd⁻¹•d⁻¹, 200 mg Mn•hd⁻¹•d⁻¹, 125 mg Cu•hd⁻¹•d⁻¹, and 12 mg Co•hd⁻¹•d⁻¹ from amino acid complexes or cobalt glucoheptonate, respectively (Availa-4[®]; Zinpro Corporation, Eden Prairie, MN), for the first 30 d of the feeding period followed by transition and finishing diets that provided 360 mg Zn•hd⁻¹•d⁻¹from an amino acid complex (Availa Zn[®]; Zinpro Corporation, Eden Prairie, MN).

Experimental diets were mixed daily using a truckmounted mixer box/delivery unit. Feed was delivered throughout the day, beginning at approximately 0700 h. Study pens were fed twice daily (50% of ration in morning, 50% of ration in afternoon). Micro-ingredients, including the respective trace mineral treatments, and medicinal feed additives (monensin and tylosin) were formulated into a dry supplement that was added into the complete starting, transition and finishing diets (Table 1). Cattle were provided the finishing diet until visual appraisal determined that a majority of cattle within a replicate possessed sufficient back fat thickness to grade at least Canadian AAA with an estimated target final shrunk BW of 660 kg as the optimal slaughter weight. Once target final BW was established, target harvest dates were determined. Based on the target slaughter date, cattle across all treatments were provided RAC (Optaflexx® Elanco Animal Health, Greenfield, IN) in the supplement, at a rate of 200 mg•hd⁻¹•d⁻¹ for the final 35 d prior to slaughter. On the final day of the study, cattle were removed from their pens prior to feeding and weighed in drafts of approximately 10 to 15 steers on a certified platform scale before being loaded and shipped from the research facility to the processing plant in Brooks, AB. Cattle were weighed, shipped and harvested by replicate group. The penscale was validated using certified weights each week prior to weighing study animals. Body weights for each draft group were summed in order to calculate final pen weight. Total final BW was multiplied by 0.96 in order to adjust for gastrointestinal fill. At the packing plant, hot carcass weight (HCW), Canadian yield grade (YG), Canadian quality

Table 1. Ingredient and nutrient composition of starting and finishing diets

<u> </u>					
	Experime	Experimental Diets a			
Ingredient (%, DM Basis	Starting	Finishing			
Dry-rolled barley	41.5	86.9			
Supplement ^a	4.8	4.8			
Chopped hay	26.1	3.1			
Barley silage	27.6	5.2			
Nutrients (%, DM Basis)					
DM	65.09	80.9			
CP	12.89	12.5			
Ca	0.98	0.64			
Р	0.30	0.37			
К	1.50	0.65			
ADF	24.1	7.97			
NDF	38.4	21.1			
NEm, Mcal/cwt	73.0	88.3			
NEg. Mcal/cwt	44.8	59.2			

^a Formulated to provide a minimum of 40 mg Mn/kg, 151 mg Fe/kg, 79 mg Zn/kg, 15 mg Cu/kg, 0.30 mg Se/kg, 5500 IU Vitamin A/kg, 550 IU Vitamin D/kg, 22 IU Vitamin E/kg, 33 g monensin /tonne, and 11 g tylosin/tonne per unit of DM in the ration. Starter supplement was formulated to provide 7 g•hd⁻¹ Availa-4[®] daily for cattle on the AATM treatment and equivalent concentration of Mn, Zn, Cu, and Co from inorganic sources for the initial 30 DOF for CON treatment. Finisher supplement formulated to provide 79 ppm Zn from either zinc sulfate or Availa-Zn[®].

grade (QG), 12th rib fat thickness, *longissimus dorsi* area (LDA), and marbling score were measured and recorded by a commercial carcass data collection service. One treatment replicate was shipped for slaughter per harvest date so that both treatments within a replicate were slaughtered with the same total DOF.

Animal health was monitored throughout the study for signs of morbidity, due to bovine respiratory disease (BRD), and other health concerns at 0700 to1400 h each day. Cattle were observed for symptoms of depression, lethargy, anorexia, and respiratory distress. Animals that were removed from the pen were treated and classified as having "undifferentiated fever" (UF) if rectal temperature was ≥ 40.6 °C. Animals that appeared to be clinically morbid and had a rectal temperature of ≤ 40.5 °C, were treated with antibiotics per feedlot SOP's and classified as "no-fever" (NF). Cattle removed from study pens for treatment were placed in a hospital pen following appropriate health management procedures. These animals were monitored daily and if disease symptoms were alleviated, cattle were returned to their respective home pens. Animals in the hospital pen were fed their respective treatment diets throughout the duration of their medical care and convalescence. If cattle placed in hospital pens did not recover, within 7 d of initial treatment, their medical condition was reviewed by animal health personnel and a decision to keep or remove the animal from study was made.

Source data were checked and validated for completeness and accuracy. Total amounts of feed delivered to each pen were summed (less orts) and daily DMI calculated based on the number of pen head days. Overall live weight gain (LWG) was calculated as the difference between final and initial pen weights with (ADG) calculated from LWG divided by number of days on feed. Feed efficiency ratio was expressed as G:F. Initial carcass weight was calculated according to Tatum et al. (2012). Carcass ADG, -G:F, and total carcass gain were calculated in a similar manner as live performance. Carcass characteristics, including dressing percentage, average Canadian QG and YG, marbling score, LDA, 12th rib fat thickness, and HCW/LDA were determined.

Experimental design was a randomized complete block with pen serving as the experimental unit. Live and carcassbased growth performance DMI, G:F, HCW and other carcass parameters were analyzed using the MIXED Procedure of SAS (version 9.0; SAS Inc., Cary, NC) with replicate (block) serving as the random effect and dietary treatment as the fixed effect in the model. Proportions of cattle grading Canada AAA, AA, A, and YG 1, 2, and 3 were analyzed as binomial distributions using the GLIMMIX procedure of SAS (version 9.0; SAS Inc., Cary, NC) with the random effect of replicate in the model. Tests of fixed effects were considered significant if $P \le 0.05$ and a tendency if $0.05 > P \le 0.10$.

RESULTS AND DISCUSSION

Mean days on feed averaged approximately 253, ranging from 239 to 262 d. Performance parameters, calculated either on a live animal or carcass basis indicated final BW, DMI, ADG, and G:F were not different between treatments (Table 2). No treatment differences (P = 0.69) were detected for initial carcass weight; however, HCW and total carcass gain were numerically greater ($P \le 0.15$) in CON animals compared to AATM. Carcass ADG was similar (P = 0.42) between treatments with no difference (P = 0.73) observed between CON and AATM for carcass G:F.

Carcass characteristics including dressing percentage are presented in Table 3. Despite numerical differences in final BW and HCW, dressing percentage for CON and AATM were similar (P = 0.97). Marbling score did not differ (P=0.50) between treatments. Fat thickness measured at the 12th rib was 4.2% lower (P = 0.19) for animals consuming AATM versus CON. Cattle receiving AATM had a numerically (P = 0.20) larger LDA than CON. The ratio of HCW:LDA as indicated by Beckett et.al., (2009) can be used to evaluate shifts in overall lean carcass tissue accretion, with commercial slaughter data. This is done by evaluating the differences in red meat accretion, relative to carcass fat often observed with the use of beta-agonists. These values (Table 3) indicated cattle receiving AATM had approximately a 2.3% lower (P = 0.03) HCW:LDA compared to those consuming CON. This was largely attributable to the numerically greater HCW of CON steers. However, calculated yield grade (YG) was improved (P = 0.08) by 5.2% in AATM suggesting a higher lean meat yielding carcass was produced by feeding AATM with no difference in the content of intramuscular fat.

The proportion of carcasses designated as high QG (Prime +AAA), AAA and A were not affected ($P \le 0.97$; Table 4) by dietary treatment. However, the proportion of carcasses designated as low QG (B4+E) grade was decreased ($P \le 0.01$) by feeding AATM throughout the feeding period compared to CON. The proportion of carcasses distributed across the three yield grade (YG) categories was markedly affected by dietary treatment. The feeding of AATM indicated a general

 Table 2. Live animal and carcass performance of crossbred

 steers fed finishing diets providing Zn from an inorganic

 source (CON) or amino acid complexed source (AATM)

	Tre	atment	_	
				P-
Item	CON	AATM	SE ^b	value
Pen, n	10	10		
Initial hd, n	2,266	2,276		
Final hd., n	2,156	2,173		
Mean days on feed	253	253		
Live performance				
Initial BW, kg	281	281	1.9	0.70
Final BW, kg	664	657	4.2	0.12
Daily gain, kg/d	1.51	1.49	0.01	0.11
DM intake, kg/d	9.32	9.20	0.06	0.18
G:F, kg	6.18	6.20	0.06	0.96
Carcass performance				
Initial carcass wt., kg	177	177	1.4	0.69
Hot carcass wt., kg	405	402	2.6	0.15
Total carcass gain, kg	228	225	3.2	0.14
Daily carcass gain, kg	0.90	0.89	0.09	0.42
Carcass G:F	0.01	0.01	0.09	0.71

^a CON= Zn, Mn, Cu and Co from inorganic sources; AATM

= Zn, Mn, Cu and Co from amino acid complex..

^b Standard error of least squares means.

Table 3. Carcass parameters of crossbred steers fed finishing diets providing Zn from an inorganic source (CON) or amino acid complexed source (AATM)

	Treat	_			
			-	P-	
Item	CON	AATM	SEb	value	
No. carcasses, n	2,005	2,061			
Dressing percentage	61.07	61.14	0.09	0.52	
Marbling score ^c	392	390	7.3	0.50	
Fat thickness at 12th rib	0.48	0.46	0.03	0.19	
Longissimus dorsi area, cm2	94.8	96.1	1.1	0.20	
HCW:LDA, kg/cm ²	4.3	4.2	0.05	0.03	
Calculated yield grade	3.02	2.86	0.11	0.08	

^a CON= Zn, Mn, Cu and Co from inorganic sources; AATM =

Zn, Mn, Cu and Co from amino acid complexes.

^b Standard error of least square means..

^c Camera visual-grade marbling score such that 300 = slight⁰⁰;

400 = small⁰⁰, 500 = modest⁰⁰

Table 4. Effects of feeding crossbred steers finishing diets providing diets Zn from an inorganic source (CON) or amino acid complexed source (AATM) on number and distribution of carcasses with Canadian yield and quality grades

	Treatment			
				P-
Item	CON	AATM	SE ^a	value
Canadian quality grades				
Total carcasses scored, n	2,005	2,060		
Prime + AAA				
No. carcasses	774	814		
% of total carcasses	38.6	39.5	0.04	0.55
AA				
No. carcasses	1,107	1,155		
% of total carcasses	55.2	56.1	0.04	0.61
A				
No. carcasses	62	63		
% of total carcasses	3.1	3.1	0.01	0.97
<u>B4+E</u>				
No. carcasses	62	28		
% of total carcasses	3.1	1.3	0.01	0.01
Canadian yield grades				
Total carcasses scored, n	2,001	2,060		
Yield grade 1				
No. carcasses	1,119	1,279		
% of total carcasses	55.9	62.1	0.05	0.01
Yield grade 2				
No. carcasses	568	520		
% of total carcasses	28.4	25.2	0.03	0.04
Yield grade 3				
No. carcasses	314	261		
% of total carcasses	15.7	12.7	0.02	0.02

^a CON= Zn, Mn, Cu and Co from inorganic sources; AATM = Zn, Mn, Cu and Co from amino acid complexes.

^b Standard error of least square means.

shift in the proportion of carcasses to that of a leaner, greater muscling carcass. The proportion of carcasses grading Canada YG 1 was greater ($P \le 0.01$) and Canada YG 3 was lower (P = 0.02), for cattle fed AATM compared to CON. Additionally, CON produced (P = 0.04) a larger proportion of carcasses grading Canada YG 2, relative to AATM. This

response indicates that carcasses from steers fed AATM were on average leaner than those from CON cattle. These data suggest that feeding AATM in combination with RAC may increase overall carcass leanness, compared with feeding RAC with a strictly inorganic source of Zn. Cell culture research (Harris et. al., 2013) indicated greater sustained level of activity for RAC when exposed to higher levels of Zn in the media. While iso-levels of dietary Zn were fed in this study, the greater relative bioavailability of Zn provided by AATM may have stimulated a greater response in the enzyme systems required for RAC activity. Additional research is required to demonstrate this response in vivo and to more fully understand the relationship which AATM, particularly Zn may have on expression and activity of BA fed to finishing cattle, particularly the dose and duration of feeding required for achieving an optimum response.

Overall morbidity, as measured by the number of animals pulled and treated for any condition ranged from 2.9 to 59.6% across all study pens (Table 5). In general, morbidity rate varied greatly by block indicating a source of origin and possibly time of arrival or location within feedlot effect. The total percentage of animals enrolled on study that subsequently received medical treatment for any cause was approximately 17.5%, with the AATM group having a lower ($P \le 0.10$) apparent overall morbidity rate compared to CON (16.5 vs.18.4% of initial head, respectively) resulting in 41 fewer animals that had to be pulled and treated for medical conditions. The total number of times an animal was pulled and medical treatment rendered was also decreased $(P \le 0.03)$ for AATM (468 hd) compared to CON (530 hd). No differences between CON and AATM were observed in apparent morbidity rate for total respiratory (BRD) cases, including both febrile and non-febrile BRD cases; joint infection cases or other non-specific or miscellaneous cases. Placing individual disease conditions into general groups of

Table 5. Effects of feeding crossbred steers finishing diets Zn from an inorganic source (CON) or amino acid complexed source (AATM) on number of animals requiring veterinary treatment

	Trea	itment ^a	-	
Item	CON	AATM	SEb	P- Value
Initial hd, n	2,266	2,276		
Total hd treated, n	411	370	0.04	0.10
Total respiratory cases, n c	86	91	0.007	0.73
Total metabolic cases, n d	70	39	0.006	0.01
Total infectious cases, n e	221	183	0.02	0.63

^a CON= Zn, Mn, Cu and Co from inorganic sources;

AATM = Zn, Mn, Cu and Co from amino acid complexes.

^b Standard error of least square means.

^c Includes sum of primary treatment cases for febrile and non-febrile respiratory disease.

^d Includes sum of primary treatment cases for urinary calculi, bloat and atypical interstitial pneumonia.

^e Includes sum of primary treatment cases for total primary respiratory (febrile and non-febrile), primary footrot and primary joint infection.
metabolic and infectious indicated no treatment effect for the number of infectious disease requiring treatment between CON and AATM, although an apparent reduction ($P \le 0.01$) in the number of animals treated for various conditions classified as metabolic, i.e., bloat, urinary calculi and AIP was observed. The number of animals requiring medical treatment for bloat and foot rot tended to be lower in steers fed AATM, as were the number animals requiring medical attention for trauma and injury ($P \le 0.04$).

Overall mortality (Table 6) averaged 3.2% across both treatments with 79 total mortalities for CON and 66 total mortalities for AATM treatment groups (P=0.29). Total mortalities due to respiratory disease were numerically higher for cattle receiving AATM compared to CON treatment, however, this was not a different (P = 0.20). Animals succumbing to mortality due to metabolic disorders, primarily bloat were numerically greater when receiving the CON versus the AATM treatment, however these percentage populations were not found to be different (P = 0.65). Mortality resulting from varied causes and generally classified as "other" was reduced ($P \le 0.04$) in the AATM group compared to CON.

 Table 6. Effects of feeding crossbred steers finishing diets Zn

 from an inorganic source (CON) or amino acid complexed

 source (AATM)on animals dying on study

	Tre	atment	_	P-
Item	CON	AATM	SE	Value
Initial hd, n	2,266	2,276		
Respiratory, n	9	16	0.001	0.20
Metabolic, n	23	20	0.002	0.65
Histophilus, n	9	10	0.001	0.83
Urinary calculi, n	12	4	0.001	0.09
Other, n	38	20	0.003	0.04
Total, n	79	66	0.004	0.29

^a CON= Zn, Mn, Cu and Co from inorganic sources; AATM = Zn, Mn, Cu and Co from amino acid complexes. ^b Standard error of least square means.

IMPLICATIONS

The response of BA and Zn amino acid complexes can be variable in terms of both nature and magnitude and as such requires further investigation to better understand the dietary, animal and other factors which may contribute to the type and level of response which may be expected in commercial use. The results of this study would suggest an AATM program may provide incremental improvements above that achieved by RAC alone. Development of data that would allow better recommendations for the timing, duration and level of feeding Zn-AATM for achieving an optimal response is required.

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Effect of dietary level of oregano essential oil on carcass characteristics of finishing hair lambs

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ABSTRACT. Oregano essential oil (OEO) is considered a plant extract capable of improving animal performance in ruminants due to its Carvacrol (CAR) content. The objective was to evaluate 3 CAR levels on hair lamb carcass characteristics: slaughter body weight (SBW), hot carcass weight (HCW), cold carcass weight (CCW), dorsal fat (DF), LM area, KPH and commercial cuts (CC). Carvacrol content in OEO was 62.7%. Lambs were randomly assigned to five treatments (TRT; DM basis): Control 0 g/kg CAR (CON), Monensin, 33mg/kg (R), CAR1, 0.2 g of CAR/kg; CAR2, 0.3g of CAR/kg; and CAR3 0.4 g of CAR/kg. Lambs were fed ad libitum for 70 d with 80:20 concentrate:forage ratio diet. Fifty crossbred hair lambs (Dorper x Pelibuey and Charolais x Pelibuey) were used in a performance trial, and only twenty lambs (four per TRT) were used to evaluate carcass characteristics. At slaughter carcasses were weighed (HCW) and stored at 4°C degree for 36 h, then carcasses were weighed again for CCW, and CC were obtained. Data was analyzed in a completely random design using lamb as the experimental unit. For SBW (kg), HCW (kg), and CCW (kg) there was no TRT effect (P > 0.05); TRT means were SBW: 46.9, 46.5, 45.03, 45.61 and 44.3; HCW: 23.3, 22.5, 21.7, 21.2 and 22.0; CCW: 22.7, 21.8, 21.1, 20.7 and 21.2, for CON, R, CAR1, CAR2 and CAR3, respectively. Dorsal fat (DF) was the only variable showing a TRT effect (P < 0.05) and CON lambs had the highest mean $(5.64 \pm 0.49 \text{ mm})$. Commercial cuts (kg) showed no TRT difference (P > 0.05), commercial cuts per treatment during the test were 3.21, 2.9, 2.8, 2.7 and 2.7; 0.82, 1, 0.97, 1.1 and 1.16; 4.7, 4.3, 3.9, 4.0 and 4.23; 2.47, 2.27, 2.36, 2.10 and 2.36, for leg, neck, short loin and shoulder for CON, R, CAR1, CAR2 and CAR3, respectively. Supplementing a high concentrate diet with different Carvacrol levels to finishing hair lambs did not caused an effect on their carcass characteristics.

Keywords: carvacrol, finishing hair lamb, carcass

INTRODUCTION

Mexico has an 8,405,902 ovine inventory (SIAP, 2014), with finishing lamb as the main product for national consumption. High concentrate feeding systems for feedlot lambs have shown an improvement in ADG, GE and carcass characteristics (Villalobos, 2010). Nowadays an important consideration in intensive production systems is the use of organic additives instead of growth promoters and antibiotics

including ionophores (Chaves et al., 2008). Essential oil compounds (EOC) have been considered an alternative to replace antibiotic additives on animal production due to their similar antibiotic effects (Benchaar et al., 2008). Benefits observed in the use of EOC are the health benefit in animals and a friendly environmental obtaining process (Aligiannis et al., 2001).

Mexican oregano (Lippia graveolens HBK) is a common native plant in arid and semiarid grasslands in northern Mexico. Due to its antimicrobial properties, oregano has been examined as an alternative growth promoter in broiler chickens (Lewis et al., 2003), pigs (Docic and Bilkei, 2003), and little information is available for growing lambs (Bampidis et al., 2005). No evidence is available on potential growth promoting effects on carcass characteristics of oregano essential oil when added in finishing lamb diets, so the objectives of this study were to examine carcass characteristics in finishing hair lambs with a diet supplemented with oregano essential oil.

MATERIALS AND METHODS

All procedures involving lambs were conducted within the guidelines of approved local official techniques for animal care in Mexico (NOM-051-ZOO-1995: Humanitarian care of animals during mobilization; NOM-033-ZOO-1995: Slaughter of domestic and wild animals). This study was conducted at the Facultad de Zootecnia y Ecologia of the Universidad Autonoma de Chihuahua, Chihuahua, Mexico.

Experimental Design and Treatments

Twenty finished hair lambs were used (averaging 45.9 \pm 2.82 kg of slaughter BW and 130 days old). Treatments consisted (DM basis): 1) 0 g/kg of Carvacrol + basal diet, BD (CON); 2) 0 g/kg of Carvacrol + 33 mg/kg of Rumensin + BD (RUM); 3) 0.2 g/kg of Carvacrol + BD (CAR1); 4) 0.3 g/kg of Carvacrol + BD (CAR3). Fifty crossbred hair lambs (Dorper x Pelibuey and Charolais x Pelibuey) were used in a performance trial, and only twenty lambs (four per TRT) were used to evaluate carcass characteristics. They were withheld from feed for 18 h and water for 12 h before slaughter, and their fasted BW was taken just before slaughter. Hot carcass weight (HCW) was determined on the day of slaughter. Carcass yield (CY)

was calculated with the formula CY= (HCW*100)/slaughter weight. Carcasses were chilled for 36 h at 4° C and cold carcass weight (CCW) was recorded. Carcasses were ribbed at the 12th rib, and LM area was measured between the 12th and 13th ribs, and 12th rib fat thickness (DF) was evaluated. Dressing percentage was estimated as (CCW*100)/slaughter weight. After dressing and storing refrigerated for 24 h at 4° C, carcasses were sectioned into two symmetric halves. The right half was divided into the cuts: neck, short loin, shoulder, and leg, and weights of each cut were recorded. Finally kidney, pelvic, and hearth fat (KPH) were evaluated as percentage of carcass.

Statistical Analyses

Data was analyzed with the PROC GLM of SAS in a completely random design, lamb was the experimental unit, and model included treatment effect.

RESULTS AND DISCUSSION

There was no TRT effect for oregano essential oil supplemented lambs (P > 0.05) in SBW (kg), TRT means (Table 1) were 46.9, 46.5, 45.03, 45.61 and 44.3 for CON, R, CAR1, CAR2 and CAR3, respectively. Simitzis et al. (2008) found similar results for SBW and dressing in lambs fed with concentrate and alfalfa hay with oregano essential oil spread over concentrate. Results reported here for HCW and CCW (Table 1) showed no differences (P > 0.05); TRT means were for HCW (kg) 23.3, 22.5, 21.7, 21.2 and 22; and for CCW (kg) 22.7, 21.8, 21.1, 20.7 and 21.2, for CON, R, CAR1, CAR2 and CAR3, respectively, and are similar to those reported by Bampidis et al. (2005), who did not found either a TRT effect by supplementing oregano leaves to finishing lambs. Chaves et al. (2008) also reported no differences for HCW in lambs supplemented with carvacrol.

Carcass yield (%) showed no TRT effect (P > 0.05), CON and CAR3 had the higher values (46.69 and 49.62 ± 1.09) and CAR2 the lowest value 46.53 (± 1.09). Dressing (%) showed no TRT effect either (Table 1). Rib eye area (cm²) was similar among TRT (P > 0.05) but R supplemented lambs showed the higher value (34.58 ± 4.64), and CAR2 had the lowest value (26.53 ± 4.64). Dorsal fat (DF) was the only variable showing a TRT effect (P < 0.05) and CON lambs had the highest mean (5.64 ±0.49 mm, Table 1) while in this case CAR1 had only 2.94 mm (±0.49), a trend for increased DF is observed for CAR levels. Similar results have been reported by Meyer et al. (2009) when supplementing steers with an essential oil mixture or rumensin plus tylosin.

For commercial cuts weight (short loin, leg, neck, and shoulder) no differences were found (P > 0.05; Table 1). Bampidis et al. (2005) reported similar results they found no differences in short loin, neck and leg weight in lambs supplemented with oregano leaves; however Chaves et al. (2008) reported differences on sirloin cap-on cut.

IMPLICATIONS

Supplementing a high concentrate diet with different Carvacrol levels to finishing hair lambs did not caused an effect on their carcass characteristics. Since it appears that changes in carcass characteristics are Carvacrol dose dependant, perhaps research with higher doses of Carvacrol is necessary, however, the cost and availability of oregano essential oil in comparison to other additives must be considered.

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Table 1. Least square means and standard error of the effect of carvacrol on carcass characteristics of finishing hair lambs

			Treatment				
Ítem	CON	R	CAR1	CAR2	CAR3	SE	P-value
Slaughter BW, kg	46.99	46.50	45.03	45.62	44.39	1.31	0.5182
HCW, kg	23.34	22.59	21.72	21.22	22.02	0.72	0.3173
CCW, kg	22.79	21.88	21.18	20.74	21.24	0.64	0.2416
Carcass yield, %	49.69	48.44	48.15	46.53	49.62	1.09	0.3072
Dressing percentage, %	48.49	46.94	46.96	45.47	47.86	6.95	0.3004
LM area, cm2	28.12	34.58	28.35	26.53	27.56	4.64	0.5093
DF, mm	5.64	3.78	2.94	3.59	3.83	0.49	0.0323
KPH, %	0.1432	0.1164	0.1556	0.2149	0.1403	0.04	0.3431
Leg, kg	3.22	2.93	2.82	2.79	2.77	0.17	0.3067
Short loin, kg	4.71	4.38	3.97	4.02	4.24	0.26	0.3040
Neck, kg	0.83	1.01	0.97	1.16	1.16	0.15	0.4399
Shoulder, kg	2.47	2.28	2.36	2.10	2.37	0.10	0.1844

CON: 0 g/kg of carvacrol + BD; R: 0 g/kg of carvacrol + 33 mg/kg of Rumensin 200[®] + BD; CAR1: 0.2 g/kg of carvacrol + BD; CAR2: 0.3 g/kg of carvacrol + BD; CAR3: 0.4 g/kg of carvacrol + BD

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Capsaicin supplementation does not reduce lipopolysaccharide-induced inflammation in growing beef steers¹

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ABSTRACT: This study evaluated effects of dietary supplementation of jalapeño powder containing capsaicin on inflammation in 24 beef steers $(213 \pm 6.2 \text{ kg BW})$ exposed to lipopolysaccharide (LPS). Treatments were a 2×2 factorial of 2 dietary supplements that supplied 0 or 0.74 ± 0.02 mg kg-1 BW of capsaicin (-CAP vs. +CAP), and 2 infusions of sterile saline that supplied 0 or 0.5 µg kg-1 BW of LPS (-LPS vs. +LPS). Steers were limit-fed a diet for 15 d, and supplemented with dietary treatments from d 8 to 15. On d 15, LPS was infused (via i.v. catheters) 3 h after feeding. Respiration rates, rectal temperatures, and blood samples were collected at 0, 2, 4, 8, 12, and 24 h after LPS infusion. Data was analyzed using mixed models and repeated measures. A CAP × LPS interaction occurred for respiration rate (P = 0.07) and serum glucose (P = 0.06). Respiration rate was greater for +LPS than -LPS steers fed -CAP, and not different for +LPS and -LPS steers fed +CAP. Glucose was not different for +LPS and -LPS steers fed -CAP, and lower for +LPS than -LPS steers fed +CAP. An LPS × h interaction (P < 0.01) occurred for all variables. Respiration rates were greater for +LPS than -LPS steers at 2 h, and not different at 4, 8, 12, and 24 h. Rectal temperatures were greater for +LPS than -LPS steers at 2 and 4 h, not different at 8 and 12 h, and lower at 24 h. Cortisol and IL-6 of +LPS steers were greater at 2, 4, 8, and 12 h (IL-6 only), and not different from -LPS steers at 24 h. Serum prolactin was greater for +LPS than -LPS steers at 2 h, lower at 8 and 12 h, and not different at 24 h. Insulin and tumor necrosis factor- α were greater for +LPS than -LPS steers at 2 h, and not different at 4, 8, 12, and 24 h. Interferon- γ was not different at 0 and 2 h, greater for +LPS than -LPS at 4 h, and not different at 8, 12, and 24 h. Serum glucose was greater for +LPS than -LPS steers at 2 h, lower at 4 h, and not different at 8, 12, and 24 h. Results demonstrated that dietary supplementation of jalapeño powder containing capsaicin did not reduce LPS-induced inflammation in steers.

INTRODUCTION

Morbidity in feedlot calves decreases performance and negatively impacts gross income (Waggoner et al., 2007). Calf morbidity is generally associated with exposure to infectious diseases, such as bovine respiratory disease complex, and is associated with stress from handling, commingling, and transportation to the feedlot. Exposure to infectious pathogens causes inflammation and stimulates physiological, nutritional, and immunological changes (Loerch and Fluharty, 1999). Sick cattle are commonly treated with antibiotics and anti-inflammatory drugs to reduce fever, however increased consumer pressure to minimize use of antibiotic and other drugs in the cattle feeding industry demands exploration of alternative strategies to improve animal health.

Capsaicin is a capsaicinoid that contributes to the pungent sensation in hot peppers. In addition to the pharmacological roles of capsaicin in pain, cardiovascular, and respiratory systems (O'Neill et al., 2012), previous research demonstrated that capsaicin may have anti-inflammatory properties. For example, Dogan et al. (2004) reported that intraperitoneal injection of capsaicin decreased the febrile response of rats exposed to lipopolysaccharide (LPS). Also, Demirbilek et al. (2004) demonstrated that capsaicin lowered pro-inflammatory cytokines (tumor necrosis factor- α , and interleukin-6) and increased anti-inflammatory cytokines (interleukin-10) in septic rats. Therefore, we hypothesized that the capsaicin in hot peppers will reduce inflammation in cattle exposed to stress and disease. The objective of this study was to evaluate effects of dietary supplementation of jalapeño powder containing capsaicin (1,280 mg/kg) on inflammation and nutrient metabolism of beef steers exposed to an endotoxin.

MATERIALS AND METHODS

Experimental Design and Treatments

Key words: capsaicin, lipopolysaccharide, steer

The Institutional Animal Care and Use Committee at New Mexico State University approved all procedures.

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Twenty-four crossbred steer calves $(213 \pm 6.2 \text{ kg} \text{ initial BW})$ were housed individually in soil-surfaced pens for the first 11 d of the study, and then were adapted to individual tiestalls of an animal metabolism facility from d 12 to 14 before collection of samples on d 15. Catheters (J457A; Jorgenson Laboratories, Loveland, CO) were placed into the jugular vein of calves on d 14. All calves had free access to water and were individually fed a basal diet (Table 1) at $1.53 \pm 0.05\%$ of BW (DM basis) in equal portions twice daily (0700 and 1900) throughout the 15-d experiment. Steers were limit-fed to represent feed intakes typical of newly received feedlot calves (NRC, 2000).

The experiment was a randomized complete block design with calves divided into 2 blocks (12 animals per block) because of a limited number of tie-stalls in the animal metabolism facility. Within each block, calves were randomly assigned to a 2×2 factorial arrangement of treatments. Treatments were 2 dietary supplements that supplied 0 or 0.74 ± 0.02 mg kg-1 BW of capsaicin (-CAP vs. +CAP), and 2 intravenous infusions of sterile saline that supplied 0 or 0.5µg kg-1 BW of LPS (-LPS vs. +LPS; E. coli 055:B5; Sigma Chem. Co., St. Louis, MO). Dietary supplements (Table 2) were formulated to be isonitrogenous, and jalapeño powder (contained 1,280 mg/kg capsaicin) partially replaced corn grain and soybean meal for the +CAP supplement. Dietary supplements were mixed with the basal diet at the 0700 and 1900 feedings from d 8 to 15 of the experiment. Steers were adapted to 200 g/d of dietary supplements from d 8 to 11, and received 400 g/d of the dietary supplements from d 12 to 15. For the -LPS and +LPS treatments, 50 mL of sterile saline solution (without or with dissolved LPS) was infused (Model 230 Syringe Pump, KD Scientific, Holliston, MA) at 1 mL/ min via jugular catheters at 3 h after the 0700 feeding on d 15. In block 1, a steer was removed from the experiment because it was dehydrated when the jugular catheter was inserted on d 14, and in block 2 a steer was removed after the 4-h blood

Table 1. Composition of the basal diet

Item	DM basis
Ingredient, % of DM	
Corn grain, cracked	38.2
Soybean hulls, pelleted	20.0
Alfalfa hay	20.0
Dried distiller's grains	10.0
Molasses	8.0
Soybean meal	2.5
Premix ¹	1.3
Nutrient, % of DM	
ADF	22.8
CP	16.3
Ca	0.77
Р	0.30

¹Supplied (DM basis): 0.50% urea, 0.35% limestone, 0.30% salt, 0.05% dicalcium phosphate, 20 mg/kg Fe, 18 mg/kg Zn, 3.6 mg/kg Cu, 3.4 mg/kg Mn, 0.07 mg/kg Se, 3,000 IU/kg vitamin A, 600 IU/kg vitamin D, 150 IU/kg vitamin E, and 33 mg/kg monensin.

collection because of a severe reaction to the +LPS infusions that warranted medical treatment.

Sample Collection and Analysis

Respiration rates and rectal temperatures were measured, and blood samples collected at 0 (immediately before), 2, 4, 8, 12, and 24 h after the infusion of LPS on d 15. Respiration rates were measured using a stethoscope and stopwatch, and rectal temperatures were measured using portable digital thermometers (ReliOn, China). Blood samples were collected via jugular catheter into 10-mL syringes, and then transferred into vacuum tubes for separation of serum (Corvac serum separator) and plasma (Monoject Sodium Heparin, Kendall, Ontario, CA). Blood samples for serum collection were allowed to coagulate for 30 min at ambient temperature, and blood samples for plasma collection were immediately placed on ice. All blood samples were centrifuged (1,500 \times g; Beckman TJ-6R Centrifuge, Palo Alto, CA) for 20 min at 10°C, and aliquots of serum and plasma were transferred into multiple vials and frozen at -20°C or -80°C for later analysis.

Serum samples were analyzed for cortisol (Kiyma et al., 2004) and insulin (Camacho et al., 2012) by solid-phase RIA with commercially available antibody-coated tube technology (Siemens Diagnostics, Los Angeles, CA), and prolactin and IGF-I by double antibody RIA as described by Spoon and Hallford (1989) and Berrie et al. (1995) with modifications reported by Camacho et al. (2012), respectively. Within and between assay CV were less than 12% for all RIA. Glucose concentrations in serum were analyzed using a commercially available hexokinase reagent (Infinity TR15241, Thermo Scientific, Waltham, MA). The cytokines, IL-6, tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ), were measured in serum using a bovine-specific multiplex sandwich ELISA kit according to manufacturer's protocol (SearchLight, Bovine Cytokine 3-Plex Assay #29-038-1-AB; Aushon Biosystems, Inc., Billerica, MA). Within and between assay CV were less than 8% for all cytokines.

Statistical Analysis

All data were analyzed statistically as a randomized complete block design using mixed models (SAS Inst. Inc., Cary, NC) and repeated measures with autoregressive orderone covariance structure. Data collection occurred over 2

Table 2. Dietary	suppleme	nts
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	Treat	ments
Item	-CAP	+CAP
Ingredient, % of DM		
Corn grain, cracked	70	45
Jalapeño powder ²	-	30
Soybean meal	20	15
Molasses	10	10
Nutrient, % of DM		
CP	17.8	18.0

¹Dietary supplements were mixed with the basal diet (Table 1) at the 0700 and 1900 feedings from d 8 to 15 of the experiment. ²Contained 1,280 mg/kg capsaicin. periods (blocks), because the metabolism facility could house only 12 animals in individual tie-stalls. Individual animal was the experimental unit. The statistical model included effects of CAP, LPS, hour, and all possible interactions of CAP, LPS, and hour. Block and calf were random. Differences among treatments were considered significant when P < 0.05.

RESULTS

No CAP \times LPS \times h interactions ($P \ge 0.66$) were observed for all response variables measured. Also, no CAP \times LPS interactions ($P \ge 0.26$) were observed for rectal temperature and serum concentrations of cortisol, prolactin, insulin, IL-6, TNF- α , and IFN- γ in steers. A tendency for a CAP \times LPS interaction occurred for respiration rate (P = 0.07) and serum glucose concentrations (P = 0.06). Respiration rate was greater for +LPS than -LPS steers (54.1 vs 43.5 ± 7.9 breaths/ min) when supplemented with -CAP, and was not different for +LPS and -LPS steers (47.8 vs 52.5 ± 7.9 breaths/min) when supplemented with +CAP. Serum glucose concentrations were not different for +LPS and -LPS steers (78.4 vs 76.9 \pm 3.4 mg/dL) when supplemented with -CAP, and were lower for +LPS than -LPS steers (71.4 vs 83.4 ± 3.4 mg/dL) when supplemented with +CAP. No CAP \times h interactions ($P \ge$ 0.15) were observed for all response variables.

An LPS × h interaction (P < 0.01) occurred for respiration rate and rectal temperature, as well as for serum concentrations of cortisol, prolactin, IL-6, TNF- α , IFN- γ , insulin, and glucose. Respiration rate (Fig. 1) increased and was greater for +LPS than –LPS steers at 2 h, then decreased and was not different between LPS treatments at 4, 8, 12,



Figure 1. Respiration rate of steers in response to intravenous infusions of bacterial lipopolysaccharide (LPS) at h 0. Treatments were a 2 × 2 factorial of 2 intravenous infusions of sterile saline that supplied 0 or 0.5 µg LPS (-LPS vs +LPS; *E. coli* 055:B5; Sigma Chem. Co., St. Louis, MO) per kg of BW, and 2 dietary supplements that supplied 0 or 0.74 ± 0.02 mg capsaicin (CAP) per kg of BW. Effects were CAP × LPS × h (P = 0.85), CAP × LPS (P = 0.07), CAP × h (P = 0.69), LPS × h (P < 0.05), CAP (P = 0.22), and LPS (P = 0.47).

and 24 h after LPS infusion. Rectal temperatures (Fig. 2) of +LPS steers increased from 0 to 2 h and were greater for +LPS than -LPS steers at 2 and 4 h (peak), then decreased and were not different at 8 and 12 h, but were lower for +LPS than -LPS steers at 24 h after LPS infusion. Serum cortisol and IL-6 concentrations of +LPS steers increased from 0 to 2 h, were greater at 2 (peak for cortisol), 4 (peak for IL-6), 8, and 12 h (for IL-6 only), and decreased and were not different from -LPS steers at 24 h after LPS infusion. Serum prolactin concentrations increased and were greater for +LPS than -LPS steers at 2 h, then decreased and were lower for +LPS than -LPS steers at 8 and 12 h, but not different at 24 h. Serum insulin and TNF- α concentrations of +LPS steers increased from 0 to 2 h, were greater than -LPS steers at 2 h, then decreased and were not different between +LPS and -LPS steers at 4, 8, 12, and 24 h after LPS infusion. Serum IFN-y were not different between +LPS and -LPS at 0 and 2 h, increased and were greater for +LPS than -LPS steers at 4 h, then decreased and were not different between treatments at 8, 12, and 24 h after LPS infusions. Serum glucose concentrations increased and were greater for +LPS than -LPS steers at 2 h, then decreased and were lower for +LPS than -LPS steers at 4 h, and were not different between LPS treatments at 8, 12, and 24 h after LPS infusions.

DISCUSSION

Lipopolysaccharide is a component of gram-negative bacterial cell walls, and has been used to induce non-infectious inflammation in cattle (Waggoner et al., 2009a, 2009b). In the current study, increases in respiration rate, rectal temperature, and serum concentrations of cortisol, IL-6, TNF- α , and IFN- γ are indicative of stress and inflammation in steers receiving LPS. Initial increases in serum concentrations of glucose in response to LPS infusion were accompanied by a brief increase in serum insulin concentrations, which in turn were likely responsible for decreased and lower serum glucose concentration at 4 h for steers exposed to LPS. These results demonstrated that LPS infusion altered energy metabolism of steers, and are consistent with those reported by Steiger et al. (1999) and Waggoner et al. (2009b). According to Spurlock (1997), uptake of glucose by peripheral tissue is inhibited by LPS resulting in transitory resistance to insulin.

Observed tendencies for interaction between CAP and LPS for respiration rates and serum glucose concentrations suggest that capsaicin may alter responses of steers to LPS infusion. However, lack of interaction between CAP and LPS for rectal temperature and pro-inflammatory cytokines indicated that capsaicin did not suppress inflammation in steers exposed to LPS. These results contrast those of Dogan et al. (2004), who reported that an intraperitoneal injection (5 mg/kg BW) of capsaicin decreased LPS-induced fever in rats during the first 4 h after LPS injection. Additionally, Demirbilek et al. (2004) demonstrated that subcutaneous injection of a single dose of capsaicin at 1 mg/kg of BW decreased the pro-inflammatory cytokines, IL-6 and TNF- α , and increased IL-10 (an anti-inflammatory cytokine) in septic rats. Dogan et al. (2004) speculated that CAP may suppress



Figure 2. Rectal temperature, serum cortisol, prolactin, insulin, IL-6, tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), and glucose concentrations of steers in response to intravenous infusions of bacterial lipopolysaccharide (LPS) at h 0. Treatments were a 2 × 2 factorial arrangement of 2 intravenous infusions of sterile saline that supplied 0 or 0.5 µg LPS (-LPS vs +LPS; *E. coli* 055:B5; Sigma Chem. Co., St. Louis, MO) per kg of BW, and 2 dietary supplements that supplied 0 or 0.74 ± 0.02 mg capsaicin (CAP) per kg of BW. Effects for rectal temperature, cortisol, prolactin, insulin, IL-6, TNF- α , IFN- γ , and glucose were CAP × LPS × h ($P \ge 0.66$), CAP × LPS ($P \ge 0.06$), CAP × h ($P \ge 0.15$), LPS × h ($P \le 0.05$), and CAP ($P \ge 0.22$); main effects of LPS (P < 0.05) for cortisol, insulin, IL-6, and TNF- α , and no effects of LPS ($P \ge 0.11$) for rectal temperature, prolactin, IFN- γ , and glucose.

production of PGE2 by macrophages in organs that process LPS. The lack of inflammatory response to capsaicin in the current study compared with positive inflammatory response observed in previous studies (Demirbilek et al., 2004; Dogan et al., 2004) may be explained by differences in the amounts and methods of capsaicin administration. In the current study, jalapeño powder was supplemented to the diet and supplied 0.74 ± 0.02 mg capsaicin per kg of BW daily for 7 d before steers were infused with LPS. Although fed for a longer period of time, the amount of capsaicin fed in the present study was lower than the amount infused into rats. Furthermore, preliminary in vitro research in our laboratory revealed that capsaicin may be partially degraded in the rumen, and Alford et al. (2014) demonstrated that supplementation of capsaicin in jalapeño powder altered rumen microbial fermentation. Therefore, it is likely that the dietary supplementation of jalapeño powder to steers resulted in a lower post-absorptive supply of capsaicin than initially anticipated. However, greater respiration rates for -LPS steers in response to capsaicin supplementation (CAP × LPS interaction) may be an indication that capsaicin affected airway smooth muscle contraction and (or) pulmonary inflammation as reported in rats (Mandal et al., 1994).

In conclusion, results of this study demonstrated that supplying capsaicin via dietary supplementation of jalapeño powder did not reduce LPS-induced inflammation in growing steers. Alternative administration routes may be required to observe the post-absorptive effects of capsaicin on inflammation in cattle.

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Effect of dietary level of cull pinto beans (Phaseolus vulgaris) on carcass characteristics of finishing hair lambs

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ABSTRACT: The objective was to evaluate the effect of three levels of cull pinto bean (CPB) on carcass characteristics of feedlot lambs. Seventy-two crossbred hair breed lambs (Dorper × Pelibuey, and Kathadin x Pelibuey) were used (36 ewe lambs and 36 wethers, 75 ± 6 d old and 18.7 ± 3.89 kg of initial BW) in a performance trial. Lambs were sorted by gender and assigned within gender to 1 of 4 BW blocks. Within each gender/BW block, three animals were assigned at random to a pen (outdoors; 2.5×2 m) thus forming 3 gender/BW pens. Then, the three pens within each gender /BW block were allocated at random to one of the three dietary treatments. Thus, there were a total of 24 pens each containing three lambs and the experimental design was a randomized block design with a factorial arrangement of 2 genders (wether, ewe) \times 3 levels of CPB (0 %, 10 %, 20 %) × 4 replicates (blocks) with pens of 3 sheep forming the experimental plots. Lambs were slaughtered after concluding the animal performance trial. One animal from each of the 24 pens (the one that represented the average BW for the pen) was used to evaluate carcass characteristics $(34 \pm 4.1 \text{ kg})$ of BW and 163 ± 6 d of age). Treatments were (DM basis): 1) no CPB (0%; *Phaseolus vulgaris*), 2) 10% CPB; and 3) 20% CPB. Data were analyzed as a randomized complete block design. Data for HCW and cold carcass weight (CCW) decreased (P = 0.02) linearly as CPB level increased, and were greater (P < 0.01) for males. Carcass yield, LM area, and dressing percentage were greater (P < 0.01) for males. Dressing percentage quadratically increased (P = 0.04) as CPB level increased. Cull pinto beans is a suitable ingredient for feedlot hair breed lambs, but the inclusion of increasing amounts of CPB level decreases HCW, and CCW.

Key words: cull pinto beans, feedlot, hair breed lambs, high concentrate diets, Phaseolus vulgaris.

INTRODUCTION

Feed price increments have driven sheep producers to look for new alternatives in animal feeding. Agricultural byproducts are alternatives to replace high priced grains. An alternative is cull pinto beans grain popularly known as kidney beans (CPB, *Phaseolus vulgaris* L). Mexico has an inventory of 8,405,902sheep (SIAP, 2014) of which the main product is finished lamb for the domestic market. Also, about 1.7 million ha are planted with beans (SIAP, 2010). Therefore, those that do not fulfill the quality standards for human consumption can be potentially used in animal feeding. So CPB represents a good source of protein, some vitamins and minerals, and complex carbohydrates. However, it also contains anti nutritional factors such as protease inhibitors and polyphenols, lectins, and phytic acid, among others (Mejia et al., 2003); causing a detrimental effect on diet quality (Díaz-Batalla et al., 2006). Nonetheless, its effect on finishing hair breed lambs fed high-concentrate diets is unknown, and feeding high concentrate diets is not a common practice in Mexico (Villalobos et al., 2006). We hypothesized that use of CPB would be a good feed alternative for hair breed lambs, and wont affect carcass characteristics. Therefore, the objective of this study was to evaluate the effect of 3 levels of CPB supplement on carcass characteristics of finishing hair breed lambs.

MATERIALS AND METHODS

All procedures involving animals were approved by local official techniques for animal care (NOM-051-ZOO-1995: Humanitarian care of animals during mobilization of animals; NOM-024-ZOO-1995: Animal health stipulations and characteristics during transportation of animals; NOM-033-ZOO-1995: Slaughter of domestic and wild animals). This study was conducted at the Facultad de Zootecnia y Ecología of the Universidad Autónoma de Chihuahua, Chihuahua city, México.

Animals and Treatments

Seventy two crossbred hair breed lambs (Dorper × Pelibuey, and Katahdin × Pelibuey) were used (36 ewe lambs and 36 wethers, 75 ± 6 d of age and 18.7 ± 3.89 kg of initial BW). At the start of the experiment, all lambs were identified, vaccinated with a 3 way clostridial vaccine (Bacterina triple bovina, Bio- ZOO S A de C V, Zapopan, Jalisco, Mexico), treated for external and internal parasites with ivermectin (Iverfull, Aranda Salud Animal, Querétaro, Querétaro, México) and received vitamin A, D and E supplement. Lambs were sorted by gender and assigned within gender to 1 of 4 BW blocks. Within each gender/ BW block, three animals were assigned at random to a pen (outdoors; 2.5×2 m) thus forming 3 gender/BW pens. Then, the three pens within each gender /BW block were allocated at random to one of the three dietary treatments. Thus, there

I able 1. Least square	means an	iu stanuaru	CITOT TOT UN	c clicci ol	cull pinto	Dealls level 0	II Carcass char	acteristics of	IIIIISIIIII nal	LIAIDOS		
		Treatments			Ğ	ender			P-value ²		Col	ntrast ³
Item	CON	LCPB	HCPB	SEM	Male	Female	SEM	TRT	Gender	TRT ×Gender	Linear	Quadratic
Initial BW, kg	18.4	17.7	18.1	1.93	18.6	17.5	1.91	09.0	0.09	0.52	0.69	0.36
Slaughter BW, kg	35.2	34.3	32.8	1.43	36.7	31.5	1.36	0.10	<0.01	0.40	0.04	0.79
HCW, kg	17.3	17.1	16.1	0.97	17.8	15.9	0.95	0.07	<0.01	0.58	0.03	0.39
CCW, kg	17.0	16.8	15.5	0.96	17.2	15.6	0.93	0.04	<0.01	0.47	0.02	0.24
Carcass Yield	49.3	49.8	48.9	0.97	50.4	48.3	0.92	0.50	<0.01	0.91	0.64	0.28
LM area, cm2	22.8	22.0	23.0	1.18	24.9	20.3	1.06	0.72	<0.01	0.35	0.85	0.43
Dressing percentage	48.3	49.0	47.3	0.92	46.8	49.6	0.88	0.05	<0.01	0.41	0.15	0.04
¹ Treatments: CON: 0%	cull pinto l	beans; LCPB	3: 10% cull p	into beans;	and HCPB	: 20% cull pint	o beans (DM ba	sis).				
² Probabilities for the tre	satment (TK	T). Jamb ge	nder (Gende	r). and inte	raction of t	reatment × laml	b gender (level >	< gender) effec				

Probabilities for the linear (Linear), and quadratic (Quadratic) effects of cull pinto bean

were a total of 24 pens each containing three lambs and the experimental design was a randomized block design with a factorial arrangement of 2 genders (wether, ewe) \times 3 levels of CPB (0 %, 10 %, 20 %) \times 4 replicates (blocks) with pens of 3 sheep forming the experimental plots. Treatments consisted (DM basis): 1) no CPB, Control (0%, *Phaseolus vulgaris L*; CON); 2) 10% CPB (LCPB); and 20% CPB (HCPB) in the total diet, with beans replacing ground sorghum grain, cottonseed meal, and corn distiller grains in the ration (Table 2).

Carcass characteristic measurements

Lambs were slaughtered after concluding the animal performance trial. One animal from each of the 24 pens (the one that represented the average BW for the pen) was used to evaluate carcass characteristics $(34 \pm 4.1 \text{ kg of BW} \text{ and } 163 \pm 6 \text{ d of age})$. Food was withheld for 18 h and water for 12 h before slaughter, and their fasted BW was taken just before slaughter. Hot carcass weight (HCW) was determined on the day of slaughter. Carcass yield (CY) was calculated with the formula CY= (HCW*100)/slaughter weight. Carcasses were chilled for 36 h at 4°C and cold carcass weight (CCW) was recorded. Carcasses were ribbed at the 12 rib, and LM area was measured between the 12th and 13th ribs. Dressing percentage was estimated as (CCW*100)/slaughter weight.

Statistical analyses

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst., Inc. Cary, NC). Pen was the experimental unit and the model included pinto beans amount. The random statement included BW block. When significant (P < 0.05) F-statistics were noted, means were separated using linear and quadratic contrast.

RESULTS AND DISCUSSION

As expected, observed slaughter BW (P = 0.04) decreased linearly with increasing CPB (CON: 35.2 ± 1.43 ; LCPB: 34.3 ± 1.43 ; HCPB 32.8 ± 1.43) and was greater (P < 0.01) for males than females (Table 1). In agreement with these results, Villalobos et al. (2010) found a linear decreased in the performance trial. Because of slaughter BW, HCW (CON: 17.3 ± 0.97 ; LCPB: 17.1 ± 0.97 ; HCPB: 16.1 ± 0.97 ; P = 0.03), and CCW

Table 2. Diet composition (% DM) for finishing hair lambs fed cull pinto bean (*Phaseolus vulgaris* L)

		Treatmer	nts
Ingredients, %	CON	LCPB	HCPB
Alfalfa hay	20.26	20.11	20.08
Cull Pinto Bean	0.00	9.99	19.99
Sorghum grain	47.45	47.33	42.25
Cottonseed meal	18.88	11.85	11.48
Corn dry distiller grain	10.13	7.66	3.13
Animal fat	2.09	1.91	1.91
Mineral premix	0.51	0.50	0.50
Sodium chloride	0.51	0.50	0.50
Calcium carbonate	0.17	0.15	0.16
Calculated chemical composition			
CP, %	17.90	17.90	17.90
ME (Mcal/kg)	2.60	2.60	2.60
Ca	0.57	0.54	0.54
Р	0.51	0.42	0.38

(CON 17.0 \pm 0.96; LCPB: 16.8 \pm 15.5; HCPB 15.5 \pm 0.96; *P* = 0.02) also decreased linearly with increasing CPB, and was greater (*P* < 0.01) for males than females (Table 1). Different results have been reported in literature, Lanza et al. (2003) evaluated three levels of *Psium sativum*, and did not find differences for HCW and CCW among treatments, during the study they did not find differences among treatments in ADG, results that influenced directly HCW and CCW. Nonetheless, animal slaughter BW in that study was lower compared with the reported in this trial.

No differences (P \ge 0.05) were found for carcass yield (CON: 49.3 ± 0.97; LCPB: 49.8 ± 0.97; HCPB: 48.9 ± 0.97), LM area (CON: 22.8 ± 1.18; LCPB: 22.0 ± 1.18; HCPB: 23 ± 1.18), and dressing percentage (CON: 48.3 ± 0.92; LCPB: 49.0 ± 0.92; HCPB: 47.3 ± 0.92), but were greater (*P* < 0.01) for males than females (Table 1). On the other hand, dressing percentage quadratically increased (*P* = 0.04) with increasing CPB (Table 1). Lanza et al. (2003) reported similar results to those found in this experiment when lambs receive other legume grains in the diet (2% lower for lambs receiving CPB). In other study, Loe et al. (2004) reported similar dressing percentage when lambs were fed with field peas. Results indicate that feeding ovine with different legume grains do not affect dressing percentage.

By other hand, Price et al. (2006) studied different inclusion level of field peas in the diet of feedlot lambs, did not find differences for LM area when it was compared with lambs that receive diets based on corn grain, results which are similar with those reported in this study. In other study, Lardy et al. (2009) did not find treatment effect for LM area when finishing steers and heifers were fed with field peas in the diet. Although, Frendrick et al. (2005), reported a linear decreased in LM area as field peas level increased in the diet of feedlot cattle.

IMPLICATIONS

Cull pinto bean may be included in diets for finishing feedlot hair breed lambs. These results indicate that CPB are a suitable substitute for a combination of sorghum grain, cottonseed meal, and corn distiller grains in a concentrate diet. However, inclusion of CPB in more than 10% in concentrate diets for lambs resulted in negative effects on carcass characteristics as occurred with the inclusion of 20% of CPB in the diet. When formulating diets, CPB cost and availability should also be considered.

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