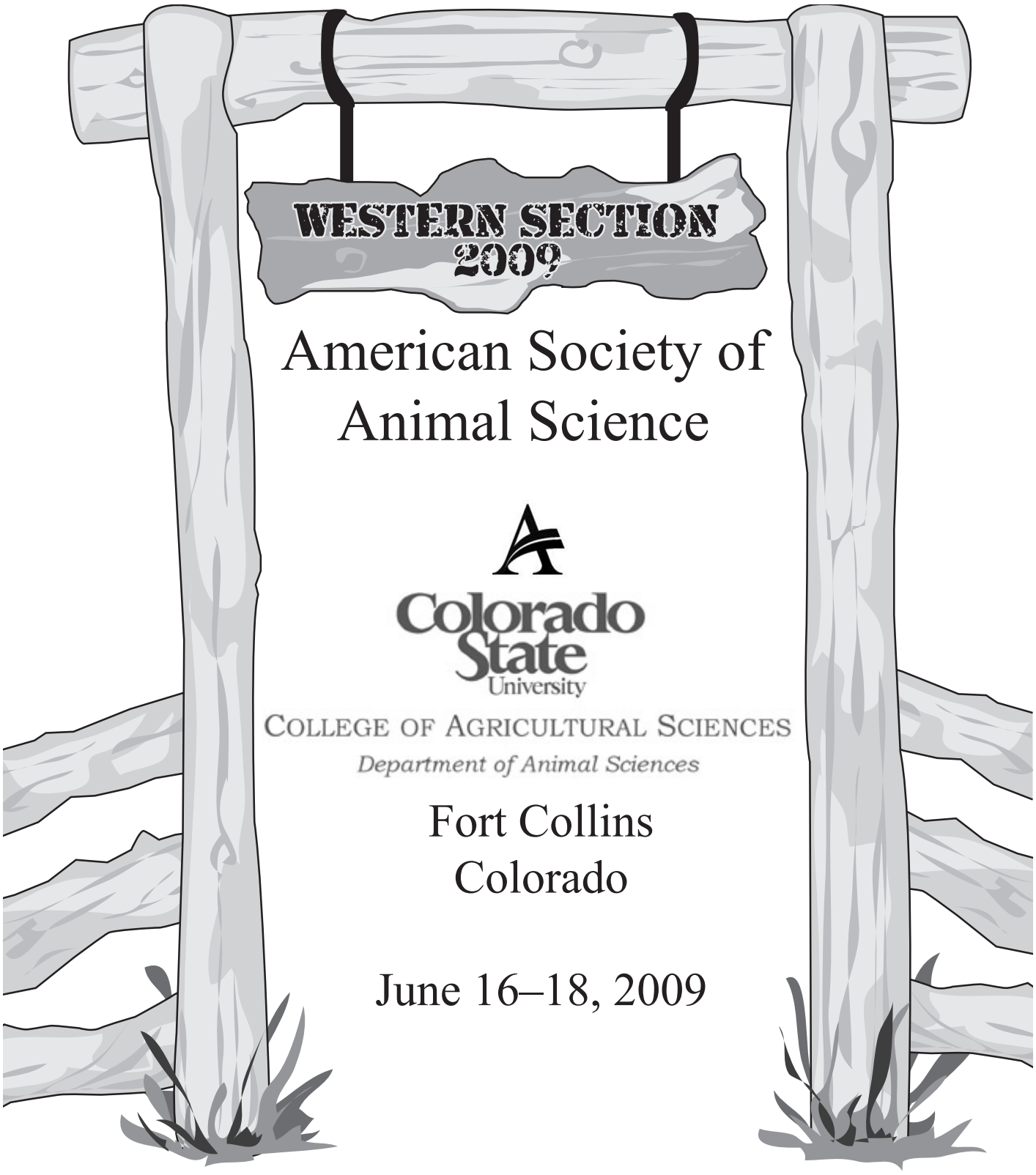
PROCEEDINGS

Volume 60



American Society of Animal Science

Colorado State University

COLLEGE OF AGRICULTURAL SCIENCES

Department of Animal Sciences

Fort Collins
Colorado

June 16–18, 2009

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WSASAS Committee Assignments 2008-2009

**** Denotes Committee Chair**

Executive

R. Battaglia, President (10, University of Idaho)**
G. Moss, President-Elect (11, University of Wyoming)
D. H. Crews, Secretary-Treasurer (12, Colorado State University)
K.C. Olson, Past-President (09, S. Dakota State University)
B. Hess, ASAS Board Director (10, University of Wyoming)
G. Tibbetts, Industry Director (09, Zinpro Corp.)
M. Salisbury (09, Angelo State University)

Awards

G. Moss, President-Elect (09, University of Wyoming)**
D. Bohnert (09, Oregon State University)
D. ZoBell (09, Utah State University)
M. Tess (09, Montana State University)
M. Du, (10, University of Wyoming)
M. Doumit (10, University of Idaho)

Symposium

T. Field (09, Colorado State University)**
T. Delcurto (09, Oregon State University)
R. Endecott (09, Montana State University)
S. Paisley (10), University of Wyoming)
J. Hall (10, University of Idaho)

Advising and Coordinating

M. Salisbury (09, Angelo State University)**
S. Ivey (09, New Mexico State University)
G. Duff (10, University of Arizona)
P. Ludden (10, University of Wyoming)
J. Bowman (10, Montana State University)
C. Mueller (10, Oregon State University)
M. Enns (10, Colorado State University)
J. Carpenter (10, University of Hawaii)
A. Roberts (10, USDA-ARS, Miles City)
J. Ahola (11, University of Idaho)
J. B. Taylor (11, USDA-ARS-USSES, Dubois, ID)
B.J. May (11, Angelo State University)

Paper Competition

J. B. Taylor (09, USDA-ARS-USSES, Dubois, ID)**
M. Shipka (09, University of Alaska)
L. Baumgard (09, University of Arizona)
K. Walburger (10, University of Saskatchewan)
K. Cammack (10, University of Wyoming)
D.L. Boss (11, Northern Ag Research Ctr, Havre, MT)
C.T. Parsons (11, Oregon State University)

Academic Quadrathlon

D.C. Rule (University of Wyoming)**
J.B. Lamb (BYU - Idaho)
S. Soto-Navarro (New Mexico State University)
R. Wiedmeier (Utah State University
H. Han (Colorado State University)

Extension

J. Sprinkle (09, University of Arizona)**
B. Bruce (09, University of Nevada, Reno)
R. Kott (10, Montana State University)
C. Parsons (10, Oregon State University)
B. Glaze (11, University of Idaho)
S. Lake (11, University of Wyoming)

Necrology

K.C. Olson, Past-President (09, S. Dakota State University)**

Nomiating

K.C. Olson, Past-President (09, S. Dakota State University)**
T.T. Ross (09, New Mexico State University)
J. Thompson (09, Oregon State University)

Minutes of the Western Section of the American Society of Animal Science Business Meeting
June 26, 2008
University of Wyoming
Laramie, WY

President Ken Olson called the meeting to order at 8:00 am.

Acceptance of the minutes of the 2007 business meeting.
The minutes of the 2007 business meeting were approved as printed in the 2008 Proceedings of the Western Section of the American Society of Animal Science.

Advisory and Coordinating Committee Report.
C.A. Loest (New Mexico State University), chair

Committee Members:

D. Drake (University of California, Davis)
R. Wiedmeier (Utah State University)
T. Bodine (Western Feed Supplements)
J. Stellflug (USDA-ARS, Dubois, ID)
D. Crews (AAFC, Edmonton)
M. Salisbury (Angelo State University)
S. Ivey (New Mexico State University)
G. Duff (University of Arizona)
P. Ludden (University of Wyoming)
J. Bowman (Montana State University)
C. Mueller (Oregon State University)
M. Enns (Colorado State University)
J. Carpenter (University of Hawaii)
A. Roberts (USDA-ARS Miles City)

On June 24, 2008, the WSASAS Executive committee requested input from the A&C committee regarding the following:

1. Graduate Student Paper Competition scoring.
   The proceedings paper and oral presentation for the Graduate Student Paper Competition are scored with equal weight, and the A&C committee evaluated a proposed scoring system that allows for the oral presentations to carry more weight than the proceedings paper. The A&C Committee meeting was held on June 25, 2008, and the committee drafted the recommendation to not change the current scoring system (the proceedings paper and oral presentation are scored with equal weight) for the graduate student paper competition. Additionally, the recommendation was made that the chair of the graduate student paper competition should provide a brief description of the scoring process prior to announcing the competition winners.

2. Criteria for sectional awards.
   The A&C committee was asked to develop criteria for the eligibility of nominees for awards. In particular, the committee was asked to evaluate the proposal that awardees should have demonstrated participation in western section activities or will be required to participate in western section activities/committees after the award. During the A&C Committee meeting on June 25, 2008, the committee drafted the recommendation that the eligibility for nominees for an award should include demonstrated participation in western section activities prior to the award, and it would be the responsibility of the awards committee to evaluate that participation. Additionally, the A&C committee recommended that participation in western section activities/committees after the award should not be required. However, the awardees should be invited to participate in western section activities.

3. Policy for publishing symposium papers.
   The A&C Committee will be working on recommendations for a draft policy statement that allows invited symposium speakers to publish symposium papers. Items to be considered include publication format (proceedings papers vs. peer-reviewed electronic JAS papers) and page charges. The final recommendations will be sent via e-mail to the WSASAS President.

4. Strategic Plan.
   The A&C committee will review the national ASAS strategic plan and will provide recommendations how the WSASAS fits the strategic plan. The recommendations will be sent via e-mail to the WSASAS President.

Academic Quadrathlon Report.
Dan C. Rule (University of Wyoming), chair

Committee Members:
J. Lamb (BYU-Idaho)
S. Soto-Navarro (New Mexico State University)
R. Wiedmeier (Utah State University)
H. Han (Colorado State University)

1. Location and date. The 2008 WSASAS Academic Quadrathlon was held in Laramie, Wyoming on the campus of the University of Wyoming on April 25th and 26th.

2. Participating universities and teams. Four universities participated in the 2008 AQ: New Mexico State University, Utah State University, BYU-Idaho, and the University of Wyoming. The teams and outcomes of the event were as follows:

Team	Event won by particular school
New Mexico State University Advisor: Dr. Sergico Soto-Navarro	Tied for 2nd in Oral Presentation 4th in Written Exam 4th in Quiz Bowl 4th in Laboratory Practicum 4th Overall
BYU-Idaho Advisor: Dr. Jim Lamb	Tied for 2nd in Oral Presentation 3rd in Written Exam 3rd in Quiz Bowl 2nd in Laboratory Practicum 3rd Overall
Utah State University Advisor: Dr. Randy Wiedmeier	1st in Written Exam 1st in Quiz Bowl 4th in Oral Presentation 3rd in Laboratory Practicum 2nd Overall
University of Wyoming Advisor: Dr. Dan Rule	1st in Oral Presentation 1st in Laboratory Practicum 2nd in Written Exam 2nd in Quiz Bowl Overall AQ Champion for 2008

Awards: The overall winning team (UW) was awarded belt buckles for each team member. The cost of the award was shared by each of the four participating universities.

No books were available to award this year.

Advisor meeting(s): Participating advisors met briefly to discuss issues of future venue and strategies to increase interest in the Academic Quadrathlon. At present, Randy Wiedmeier will contact the campus in Cedar City, Utah about holding the Western Section AQ there. This will provide a more centralized location for all teams currently participating.

Respectfully submitted,
Dan Rule

Awards Committee Report.
R. Battaglia (University of Idaho), chair

Committee Members:
T. Engle (Colorado State University)
C. Mathis (New Mexico State University)
D. Bohnert (Oregon State University)
D. Zobell (Utah State University)
M. Tess (Montana State University)

Distinguished Teacher Award

Recipient:	Dr. Paul A. Ludden University of Wyoming
Sponsor:	Elanco Animal Health
Nominator:	Dr. Douglas L. Hixon

Young Scientist Award

Recipient:	Dr. Min Du University of Wyoming
Sponsor:	Ridley Block Operations
Nominator:	Dr. Douglas L. Hixon

Extension Award

Recipient:	Dr. J. Benton Glaze University of Idaho
Sponsor:	Fort Dodge Animal Health
Nominators:	Dr. Carl W. Hunt and Dr. Richard A. Battaglia

Distinguished Service Award

Recipient:	Dr. David Ames Colorado State University
Sponsor:	DSM Nutritional Products, Inc.
Nominators:	Dr. Dr. Jack Whittier and Dr. William R. Wailes

Awards Chair, Battaglia acknowledged successful efforts to garner nominations in all categories, and encouraged early effort for 2009 nominations.

Applied Animal Science Award Report.

Graduate Student Paper Competition Committee Report.
A. Ahmadzadeh (University of Idaho), chair

Committee Members:
J. Rumph (Montana State University)
S. Soto-Navarro (New Mexico State University)
M. Shipka (University of Alaska)
B. Taylor (USDA-ARS, Dubois, ID)
L. Baumgard (University of Arizona)
K. Walburger (University of Saskatchewan)
K. Cammack (University of Wyoming)

For the 2008 WSASAS meeting 11 abstracts were originally submitted for the graduate student competition. Nine abstracts were considered for the competition and full papers were submitted prior to the dead line in April 2008. Nine graduate students representing six universities, including New Mexico State Univ., Univ. of Wyoming, Oklahoma State Univ., Univ. of Idaho, Colorado State Univ., and Univ. of

California, Davis are participating in the event.
The 2008 GSP committee consisted of eight judges, Bret Taylor, Janice Rumph, Ken Walburger; Kristi Marie Cammack, Lance Baumgard, Sergioa Soto-Navarro, Milan Shipka, and Amin Ahmadzadeh. All abstracts and papers were evaluated by all the committee members; however, due to the time conflict and prior obligations two members (Janice Rumph and Lance Baumgard) are unable to attend the 2008 WSASAS meeting and evaluate the oral presentations. We felt that six judges are sufficient to evaluate the oral presentation and thus no substitute was selected.
At the end of2008 WSASSA meeting two members, Amin Ahmadzadeh and Janice Rumph, will be completing their three-year term and thus two new members should be identified to serve in this committee. Janice Rumph has nominated Dr. Darrin Boss as her replacement. Dr. Boss is a ruminant nutrition at the Northern Ag. Research Center for Montana State and has shown interest to serve in this committee. If you have any individual who is interested to serve in this committee, please submit their names.
As a new item, the GSP Competition Committee would like the WSASAS Executive Committee to consider the following item. Based on the committee's observations of students' scores and ranking during the last two years, the GSP committee recommends that the weighting scores of the written and oral presentations to be changed so that more points should be considered for the oral presentation. The rational and justification for such recommendation will be presented by the chair of this committee to the ASASAS Executive Committee on Tuesday 6/24/08.

Extension Committee Report.
J. Ahola (University of Idaho), chair

Committee Members:

S. Paisely (University of Wyoming)
J. Paterson (Montana State University)
B. Bruce (University of Nevada – Reno)
J. Sprinkle (University of Arizona)
R. Kott (Montana State University)
C. Parsons (Oregon State University)

The Extension Committee for met via a conference call and generated a theme, topics, and speakers for a symposium to provide WSASAS meeting attendees with information about development of successful integrated research and extension programs. Topics included the availability of federal funds to support integrated research-extension programs, an overview of a model integrated program, and new and unique outreach methods to include in integrated programs.

2008 Extension Symposium Program

Wednesday June 25, 2008, 1:00 PM
Essential components for development, implementation, and evaluation of integrated research and extension proposals submitted to the USDA National Research Initiative (NRI) Competitive Grants Program.
Paper presented by: Mark A. Mirando, Competitive Programs, Cooperative State Research, Education, and Extension Service, Washington, DC

1:40 PM
New Mexico Range Improvement Task Force: An integrated team.
Paper presented by: Terrell "Red" Baker, New Mexico State University, Las Cruces

2:20 PM
Successful extension programming examples in Wyoming.
Paper presented by: Dallas Mount, University of Wyoming, Wheatland

3:00 PM
Panel discussion: What factors are critical for an integrated research and extension program to be successful? An interactive panel with discussion with USDA-NRI, extension, and research perspectives.

Beef Symposium Report.
J.B. Glaze (University of Idaho), chair

Committee Members:
B. Christensen (Virtus Nutrition)
R. Waterman (USDA-ARS Miles City)
T. Delcurto (Oregon State University)
T. Field (Colorado State University)
R. Endecott (Montana State University)

WSASAS Symposium Program:

Addressing High Input Costs in the Beef Cattle Industry: Striving for Greater Efficiency
WSASAS Beef Symposium
University of Wyoming, Laramie, WY
Tuesday, June 24, 2008
ARPAS: 4 CEUs

8:30 - 9:30 AM *Registration*

9:30 – 9:45 AM *Welcome and Introductions*
Kenneth C. Olson, Department of Animal & Range Sciences, South Dakota State University, WSASAS President
Douglas Hixon, Department Head, Department of Animal Sciences, University of Wyoming
J. Benton Glaze, Jr., Department of Animal & Veterinary Sciences, University of Idaho, WSASAS Symposium Chair.

9:45 – 10:30 AM *The Beef Industry in Transition*
Tom Field, Department of Animal Sciences, Colorado State University

10:30 – 11:15 AM *Matching Genetics with Feedstuffs in an Era of Increasing Feed Costs*
Michael D. MacNeill, USDA-ARS, Miles City, Montana

11:15 – 12:00 PM *Fitting Feed Efficiency into the Beef Profit Equation*
Gordon Carstens, Department of Animal Sciences, Texas A&M University

12:00 - 1:30 PM Lunch

1:30 – 2:15 PM *The Effect of High Input Costs on the Cattle Feeding Industry*
Tom Brink, Five Rivers Ranch Cattle Feeding, LLC, Loveland, Colorado

2:15 – 2:30 PM Break

2:30 – 3:15 PM *The Effect of High Input Costs on Cow-Calf Producers*
Chip Ramsay, Rex Ranch, Ashby, Nebraska

3:15 – 4:00 PM *Questions, Answers, and Discussion*
Speaker Panel

WSASAS Beef Symposium – Webinar: The ASAS utilized the 2008 WSASAS Symposium as a test case for presenting symposia as webinars. Webinar participants were required to pay the same registration fee ($85) as individuals registering the day of the symposium. Approximately 18 registrations were submitted for the webinar, one of which was a group of approximately 12. Number of individuals participating via the webinar was approximately 30.

WSASAS Symposium Budget: (based on figures available 07/11/08)

Income	
Registration (64 @ $65, 38 @ $85)	$ 7390.00
National ASAS Office (2 @ $1,500)	$ 3000.00
Webinar Revenue	223.08
Total	$10,613.08

Estimated Expenses	
Speaker Costs (Hotel, Mileage, Meals, etc.)	$ 2,253.50
Symposium Breaks (100 @ $8.50, 125 @ $8.50)	$ 1,912.50
Conference Room	$ 1,000.00
AV Equipment/Services	$ 500.00
Total Expenses	$ 5,666.00

Nominating Committee Report.
T.T. Ross, chair

Committee Members:
J. Thompson (Oregon State University)
P. Hatfield (Montana State University)

Necrology Report.
T.T. Ross, chair, Past-President

The following members passed away since our last meeting in June 2007.

Dr. Tim Stanton, Colorado State University
Dr. Lee Baldwin, University of California, Davis
Dr. Eric Bradford, University of California, Davis
Dr. Ward William Repp, New Mexico State University
Dr. Borden Els, New Mexico State University
Dr. Bob Raleigh, Oregon State University

Let us always remember the friendships and contributions of our passed colleagues.

Dr. Mark Healy, Utah State University, and Roy Wallace, Select Sires, were not members but certainly contributed to the Western Section in other meaningful ways.

2008 Meeting Report
G. Moss

Attendance at the Beef Symposium plus the annual meeting totaled 200 registered participants. Abstracts for the annual meeting totaled 56 oral and 46 poster presentations.

Financial Report
G. Moss

American Society of Animal Science Western Section
Financial Report Audited December 31, 2007

Balance as of December 31, 2007		**63,813.37**
Revenue and support		
Donations – General	1,100.00	
Donations – Awards	3,400.00	
Donations – Symposium	6,280.00	
Meeting Registrations	22,900.00	
Ticketed Events		
Proceedings	6,271.00	
ASAS-Symposium Support	1,500.00	
ASAS-Dues	1,130.00	
Interest Income	2,479.77	
Miscellaneous Income		
Total Revenue and Support		**45,060.77**
Expense		
Program	337.92	
Call for Papers/Abstracts	-	
Awards/Plaques	5,117.51	
Quadrathalon	2,600.00	
Convention Fees	25,196.75	
Proceedings	4,692.09	
Postage/Supplies	77.12	
Symposium Expense	2,541.28	
Travel-Speaker	-	
Travel	493.86	
Telephone	-	
Miscellaneous	1,510.00	
Staff Support	3,652.24	
Total Expenses		**46,219.20**
Net Revenue over Expense		**(1,158.43)**
Balance as of December 31, 2007		**62,654.94**

Resolutions

1. Procedure for reviewing graduate student competition abstracts.
2. Altering the scoring system for the Graduate Student Competition

ASAS Reports

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

New Business

Ken Olson passed the gavel to Dick Battaglia. Dick thanked Ken for his service to the section and presented him with the past president's plaque.

Meeting was adjourned at 9:00 am by President Battaglia.

Proceedings, Western Section, American Society Animal Science

Vol. 60, 2009

MAINTAINING BALANCE WHEN SHIFT HAPPENS

D. R. Ames
Department of Animal Sciences, Colorado State University, Fort Collins

Introduction

Congress responded to the needs of the people and initiated the Land Grant Colleges when the Morrill Act was passed in 1862. The Morrill Act designated land to each state for the purpose of education in agriculture and mechanical arts. The Hatch Act of 1887 that supports research and discovery and the Smith Lever Act of 1914 that provides for outreach, i.e. extension, were added and the Land Grant system was complete. The legislative process took 52 years to complete but the result was a balanced and comprehensive system that served agriculture and the people who lived on the land. During the recession of the 1920s and the collapse of the stock market in 1929 the Land Grant system survived because it continued to serve stakeholders. Extension was highly valued in the post depression era and Willham (2008) described major research successes such as artificial insemination, animal breeding, and nutrition during this period. Land Grant colleges (often designated A&M) were balanced programmatically with teaching, research and extension. Following World War II enrollment in Land Grant colleges soared as many colleges dropped the A&M designation and became universities. Concern for "feeding the world" resulted in the green revolution and the Land Grant University with their balance of teaching, research, and extension flourished. Post war globalization led to the addition of international programs to the mix. College Deans and Department Heads responded to stakeholders who became active advocates and advisors for Colleges of Agriculture. Animal Science Departments grew in student numbers and in stature. For a more complete history of the aforementioned changes see the pictorial history of the American Society of Animal Science (Willham, 2008). The ability to change and yet maintain balanced programs and responsiveness to stakeholders required capable administrators as leaders that were diligent supporters of the Land Grant mission.

This presentation will describe major shifts and responses to these shifts that have affected Animal Science Departments during the past 30 years. Many responses to the changing landscape have been positive; some may have been negative. Only history can say for sure. The "acid test" for evaluating the impact of decisions should focus on (1) improvement in service to stakeholders (including students) and (2) compromising program balance implicit to the Land Grant Mission.

A Lesson on Balance

In a speech to a group of fellow cattlemen relative to management techniques that had added sustainability and profit to his ranching operation, a rancher who was not an accomplished speaker drew the attention of the audience when he began by discussing balance. Everyone in attendance had expected to hear about new ways to use artificial insemination or methods to reduce input costs, but his message did not refer to techniques. Instead, he discussed interactions with his banker, nutritionist, veterinarian, extension specialist and family. After considering all inputs, he finalized his decision by incorporating a BALANCE of all advice. He was successful in raising a family while paying bills and purchasing the ranch. Never underestimate the lessons learned from clientele.

Shifts That Have Happened

The University landscape changed dramatically during the 1980s and 1990s. There were new technologies, uncharted issues and new paradigms. Some challenges facing the Land Grant system in general and Animal Science Departments in particular during the past three decades included the following.

Electronic Technology. Consider the fact that the internet, email, word processing and a PC on every desk only began in the 1980's and that rapid increases in computer dependence did not happen until the mid 1990's.

Reduced Funding. Budget rescissions have become commonplace. Most states reduced support in 1982 and 1986 and the impact of "911" and the recession of 2008 have caused serious reductions in appropriated funds.

*Demographics***.** Urban population in the West has increased. Small acreages have increased and fewer

incoming students have hands-on experience with food animals.

New Issues. All eras of food animal production have dealt with issues. However, following the green revolution (when concern for the capacity to produce food diminished) many highly funded activist groups now target the food animal industry. Many academic units have developed programs that focus on food safety, animal care and environment.

*Politics***.** The political scene has had an increasingly greater impact on animal agriculture during the past 30 years. Professional societies, clientele groups and universities now have PACs and/or lobbyists to liaison with state and federal government.

Because of these issues, administrators have responded with a variety of decisions. The challenge should be to deal with difficult issues without losing site of the goal: namely, to maintain balanced programs and serve stakeholders.

Maintaining Balance When Shift Happens

Land Grant institutions should be dynamic but the principle of balanced teaching, research, and extension programs to serve stakeholders must be maintained. Strategic planning and change has been a popular theme during the past three decades. We have witnessed "change merchants" in university administration that jump on any bandwagon labeled as change. Change almost became a competition among some administrators. In some cases, it appeared that there was little concern for impact of decisions on maintaining balanced programs and serving the best interests of stakeholders. Dealing with the issues listed earlier requires leadership with vision and understanding. It requires leadership that considers input from students, faculty, staff and most of all from stakeholders. Administrative responses to shifts are described below.

Adopting electronic technology. Moving rapidly to electronic communications in all phases of university programs was not a choice; it was a mandate by society! It happened quickly. The first year email addresses were published in the Colorado State University (**CSU**) Directory was 1992-93! Moreover, in a relatively short time faculty, students, administrators, staff and stakeholders now use electronic communications for a multitude of purposes. We submit research reports, search for research articles, enroll students, use Web CT and deliver information to clientele on a regular basis using electronic media. Classrooms are equipped with computer terminals where instructors use PowerPoint™, video and web generated teaching aids. Email is the standard for letters and legal correspondence to students. Electronic technologies appear to have improved program balance and successfully served stakeholders.

Dealing with Reduced Funding. Funding reduction is perhaps the most difficult issue faced by higher administration. The general approach has been to increase revenues by increasing research grants, tuition and fund raising and to reduce costs wherever possible. These approaches for solving fiscal problems all have the potential to create imbalances in programs and reductions in service to stakeholders.

With the recession of 1982, budget issues at CSU were evident. In 1981 the Dean first imitated a switch from 12 to 9-month appointments as a tool to buffer budget cuts. Of course, administrators remained 12-month employees! For Animal Science departments the 9-month appointment was first adopted at the University of Illinois and true to form, many Deans jumped on board. The original selling points were (1) less opposition from academic departments across campus, (2) that agriculture faculty would receive a 9-month salary equal to their 12-month salary and (3) more grant dollars would result because faculty could increase annual income with two additional month's salary from external funds! However, many universities did not convert from 12 to 9 with the same salary and extension faculty were also an issue. In 1990, CSU held an Ag Department Head's retreat to discuss a survey from 19 Land Grant universities relative to converting from 12 to 9-month appointments. Most of the 19 surveyed universities' representatives did not support conversion. Extension faculty and limits on the federal retirement system, ability to serve stakeholders and finding summer funding were the most common concerns listed. However, by the mid 90s many new appointments at CSU were 9-month. Currently CSU mandates 9-month appointments for all new faculty in agriculture including extension. Concerns with summer funding (after 2 years of full funding) takes precedent over serving stakeholders. This is understandable as they have families to support. Anticipated increase in grant dollars (and indirect cost recoveries) is the major factor now driving the 9-month system. In a telephone survey of 10 WSASAS Animal Science Departments, only CSU currently mandates 9-month appointments for all new faculty although several reported a variety of appointment types depending on source of funds.

Many of the changes that have occurred in the past 3 decades are based on increasing external grants and the assumption that this will result in increased indirect cost recovery. University administration view indirect costs (often-termed overhead or facility and administration

costs) as "free money" or "profit" that they can use to meet budget shortfalls or use to fund special initiatives. However, indirect costs do not represent profit. Instead, indirect costs are the audited real costs of doing research. The University is required to develop an indirect cost rate schedule that is submitted to the Department of Health and Human Services (DHHS) for review. The DHHS is the federal agency that audits costs and works with Universities to establish rates. Typical indirect cost rates negotiated with DHHS are 45-50% but the record of recovery in many universities is often less that 20%. This low recovery rate is particularly true for Departments of Animal Science where clientele organizations and USDA grants mandate zero or indirect cost rates lower than the 45-50% negotiated rates. Which administrative units on campus pay the difference in the audited cost rate and the actual recovery rate? It is safe to assume that departments who conduct the research often pay much of this difference. It has been suggested that since full indirect costs are not being recovered that funded research may actually increase university budget problems. The possibility of tuition dollars being used to support research has sparked spirited debate. Lehming (1997) addressed this issue from the National Science Foundation perspective and found that indeed the shortfall in indirect recovery could force other university resources (including student tuition dollars) to cover research costs. Grossman and Leroux (1996) cited a study in Rhode Island that questioned the role of research in tuition increases and Straayer (2008) contends that the CSU administration "spent instructional funds to scout for research money" and has "driven up tuition and fees and relied heavily upon instructional funds to expand non-instructional ventures".

A relatively new expectation of new faculty is to receive start-up monies. In the recent past, availability of computers, laboratories, livestock and equipment were provided. Now $200,000 packages for new faculty are common and figures as high as one million dollars have been reported. Has anyone calculated the payback? For example, assume actual indirect cost recovery is 18% and the departmental unit receives 22% of the indirect costs recovered which would be typical values for a Western Land Grant University. That is about 4% cost recovery at the departmental level. Using this scenario, new faculty would have to generate $5 million to recover a start-up fund of $200,000. Because of relatively low indirect cost rates permitted by clientele organizations and USDA it would require even higher levels of funding to recover start-up funds. This is only an example and often a portion of start-up monies are from central sources. However, all dollars could have been used to support other programs so it is fair to question the impact of using available funds for start-up packages designed to increase extramural research. Does this practice enhance program balance and do stakeholders benefit?

The difference between leadership and management may be defined as follows. Leadership is setting a new direction or vision for a group to follow, whereas management is controlling or directing people and resources in a group according to established directions. Deans and other administrators are expected to be strong leaders. Recently, Deans (in at least some universities) have assumed expanded roles as managers. For example, the Dean centralizes accounting activities in the Dean's office at CSU for more direct and central management. Moreover, accounting staff are more involved in programmatic decisions. At CSU centralization of all business activities in the College Dean's office was adopted to "improve efficiency" but the result is a 63% increase in accounting staff in the college as a whole. Administrators have replaced faculty in decisions on position needs and hiring decisions. It is obvious that Deans prefer candidates with a record of success in extramural funding and these preferences are evident in position announcements. Faculty annual evaluation, promotion, and tenure documents also emphasize evidence of extramural funding with less emphasis on teaching and extension, which almost certainly damages balance. In some Animal Science Departments, the Dean now controls and manages livestock units. Most colleges report increased commitment to fund raising, which may be necessary, and an important component of covering shortfalls in appropriations. Deans should be leaders not managers.

In summary, there is no doubt that the level of reduced funding dictates change. Clearly, higher administration has emphasized extramural funding in order to increase indirect cost recovery and with the hope of offsetting budget shortfalls. There are distinct advantages of securing extramural funds including enhanced graduate training and information discovery. However, emphasis on external grants and indirect costs recovery may erode balanced teaching, research and extension programs, which compromises the Land Grant Mission and diminishes the focus on serving stakeholders.

Demographics. According to U.S. Census data, there has been a steady shift toward urban from rural populations in the western states. There are more small acreages and consumers of food products have become a focus of agricultural programming. Our stakeholders are no longer limited to production agriculture but have expanded and now include many who do not make a

living from agricultural enterprises. For example, with issues dealing with food safety consumers are now stakeholders and farms operations with less than $10,000 annual sales are increasingly more important. Student demographics in animal sciences have changed drastically in the past three decades (Hallford, 2003). Reduced funding combined with expanding and more diversified stakeholders are challenging problems but the science-based information we provide does not vary. Animal Science Departments have met the challenge by adapting systems of delivery to an expanded clientele.

New issues. Animal science programs throughout the West have changed. For example, CSU hired Dr. Temple Grandin in 1990 to provide expertise in animal care, developed the Center for Red Meat safety in 1992 and recently added a new faculty member in livestock waste management. These types of shifts in expertise are evident in many Universities. Clearly, Animal Science departments have responded to our increasingly varied stakeholders by adding new programs and expertise.

Politics. The typical animal scientist does not enjoy politics except to complain when political decisions have a negative impact on animal agriculture. Fortunately or unfortunately (depending on your point of view) our profession has become highly involved in the political process during the past three decades. For example, most clientele organizations have Political Action Committees (PACs) and lobbyists in Washington. Universities employ lobbyists and our professional society has focused on the "Washington Scene" since the early 1990's. Much of the political activity has dealt with appropriations but the increase in highly funded activist groups is an area we have dealt with by providing science-based information. It is difficult to measure political success. Animal agriculture has lost funding, government regulations have increased and a multitude of inaccurate claims about the food we produce exists. However, what would have happened without influence in Washington? As stated in the movie Young Frankenstein "It could have been worse!"

Summary

Dr. James Meyer, animal scientist and former president of University of California, has written a white paper (Meyer, 2000) dealing with the Land Grant University. He recognizes the challenges facing the Land Grant system but in a series of recommendations calls for strong and consistent leadership (he believes the College Dean is pivotal) that maintains balanced roles of teaching, research and extension with a goal of serving stakeholders. An analysis of decisions made during the 1980-2010 time period will require a retrospective view and should be scheduled for the 2020 WSASAS meeting.

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EFFECT OF RU486 ON DEVELOPMENT OF SEXUAL BEHAVIOR, TESTOSTERONE SECRETION, AND EXPRESSION OF ESTROGEN RECEPTOR – β IN TWIN-BORN MALE LAMBS

P. Singh, K. J. Austin, V. A. Uthlaut, G. E. Moss, B. M. Alexander
Department of Animal Science, University of Wyoming, Laramie, Wyoming

ABSTRACT: Progesterone influences the development and expression of male sexual behavior in rodents and may be important for the expression of sexual behavior in rams. Masculinization and defeminization of the central nervous system in sheep occurs around d 60 to 70 of pregnancy. A second phase of testosterone-responsive sexual development occurs at 6 to 8 weeks of age in ram lambs. The objective of the current experiment was to determine if adult sexual behavior is influenced by progesterone during the second phase of testosterone responsive sexual differentiation. Twin born male lambs (n = 10) were used in this study. One lamb from each pair was treated with 10 mg of the progesterone receptor antagonist RU486 (n = 5), and his co-sibling was treated with an equal volume of vehicle (n = 5) twice daily from 4 to 8 wk of age. Serum concentrations of testosterone were decreased (P = 0.06) in RU486 treated rams at 9 mo, but did not differ (P = 0.5) at 18 mo of age. Investigatory behavior at 9 mo of age was decreased (P = 0.03) by RU486 treatment at the first exposure to estrous ewes, but consummatory behavior did not differ ($P \geq 0.24$). By 18 mo of age sexual behavior was not (investigatory P= 0.6, consummatory P = 0.4) influenced by treatment. Expression of steroid receptors was evaluated in hypothalamic and amygdala tissues collected at 18 mo of age. Amygdala expression of estrogen receptor-β tended (P = 0.06) to be increased in RU486 treated rams. Hypothalamic expression of progesterone receptor (PR), estrogen receptors-α –β, and androgen receptor did not differ ($P \geq 0.3$) among treatments. Sexual behavior is highly variable in males of all species. Blocking the PR during postnatal sexual development decreased sexual behavior when adolescent rams were first exposed to estrous ewes. Subsequent behaviors may be a result of an incomplete inhibition of the PR during development or increased positive stimuli provided by the rewarding aspects of sexual behavior.

Introduction

The profitability of any commercial sheep operation is dependent upon reproductive efficiency. Fitzgerald and Perkins (1993) stated that the incidence of sexually inactive rams ranges from 16 to 25% in the ram flock at the U.S. Sheep Experiment Station. In most populations of animals, sexual selection is based on the ability of males to gain access to and inseminate females. Males that have relatively poor libido or mating competence leave fewer offspring for future generations (Price, 1987). However, many animal producers tolerate slow- performing males as long as they eventually impregnate a reasonable percentage of females. Price (1987) suggested that, if tested, the number of rams culled due to mating behavior deficiencies would be as significant as the numbers currently culled for poor semen quality or physical limitations. Typical breeding practices utilize limited numbers of males to inseminate large numbers of females. Therefore, it is critical that libido (sexual interest or motivation), mating competence (ability to inseminate females), and fertility (semen quality) of males is adequate to insure reproductive success. Libido in rams is highly variable and is influenced by developmental (Roselli et al., 2003) and environmental (Price, 1987) factors.

The facilitory and inhibitory effects progesterone exerts on female reproductive behavior are well documented (Blaustein and Erskine, 2002). Progesterone is a precursor for both androgen and estrogen synthesis. In the male, androgens are necessary for the development of secondary sex characteristics and testosterone is considered the primary male sex hormone. However, the role of testosterone in the expression of male-typical behavior may be overstated since there is little correlation between plasma testosterone concentrations and male behavior (Andersen and Tufik, 2006).

Outside of its role as a precursor for androgen synthesis, the physiological significance of progesterone in the male is not well understood. Sexual differentiation in sheep occurs from approximately d 30 to 100 of the 145-d gestation (Short, 1974).The progesterone-receptor is upregulated in the hypothalamic-preoptic area (HPOA) and amygdala (AMYG) of male fetuses compared to female fetuses during brain sexual differentiation (Roselli, et. al., 2006) and may play a role in the development of central pathways necessary for the expression of adult sexual. A second phase of testosterone-responsive sexual development occurs in male sheep at 6 to 8 weeks of age (Orgeur and Signoret, 1984). Although expression of progesterone receptor in the sheep brain has not been evaluated during this neonatal developmental period, progesterone acting through its receptor may affect the expression of adult sexual behavior.

The current study examined the effect of blocking the progesterone receptor during post-natal differentiation on the

development of adult male sexual behavior and testosterone secretion.

Materials and Methods

Animal care and use was approved by the University of Wyoming Animal Care and Use Committee. Twin born male lambs (n = 10) 4 wk of age were used for this study. One sibling of each pair was treated with 10 mg of the progesterone receptor antagonist mifepristone (**RU486**; n = 5) and its co-sibling was treated with an equal volume of vehicle (n = 5) twice daily from 4 to 8 wk of age. At the end of the treatment period, lambs were weaned and fed a forage-based diet which supported moderate growth for 7 mo.

Rams were individually exposed to two ewes in estrus on three occasions at 9 and 18 mo of age. Behavior was monitored by digital camera for 30 minutes during each exposure period. Behaviors were quantified and classified as investigatory (ano-genital sniffs, flehmen, fore-leg kick, and nudge) and consummatory (mount attempt, mount, and ejaculation).

Ovariectomized ewes were induced into estrus with a 14 d exposure to progesterone using an intravaginal progesterone release device (CIDR) containing 0.3 g progesterone (InterAg, Hamilton, NZ). Following CIDR removal, ewes were treated daily with 50 μg of estradiol (i.m). Estrus was evident by 48 hr following initial estradiol treatment. Receptive behavior was maintained for five days by daily treatment with estradiol. Estrous behavior was confirmed by mature rams with known breeding competence.

Blood was collected by jugular venipuncture at 9 and 18 mo of age for analysis of serum concentrations of testosterone. Concentrations of serum testosterone were determined in a single radioimmunoassay. Antibody coated tubes and radiolabeled testosterone were purchased from Diagnostic Products Corporation (Los Angeles, CA). Standards were prepared by serial dilutions of a stock solution in charcoal treated whether serum. Assay tubes were incubated at room temperature for 4 hours. Intra-assay coefficient of variation was <10%.

Rams were killed at 18 mo of age and brains were removed from the ram's cranium and dissected using surface landmarks. The hypothalamus was separated into the preoptic area (HPOA, hypothalamic tissue dorsal to the optic chiasm) and ventral medial hypothalamus (VMH, tissue posterior to the optic chiasm and anterior of the mammillary bodies and dorsal to the roof of the third ventricle). The amygdala (AMYG) consisted of tissue from the ventromedial temporal lobe with approximately the same rostral caudal dimension of the hypothalamus containing entorhinal cortex as well as the major cortical, medial and basal amygdaloid nuclei. Tissue was maintained at -80ºC until RNA extraction.

RNA Isolation. Samples of brain tissues (100 mg) were homogenized in 1 mL of TRI reagent, incubated for 10 min at room temperature and centrifuged for fifteen minutes at 4ºC. The aqueous layer was mixed with 0.5 mL of isopropanol and the RNA was pelleted by centrifugation. The RNA was further purified using an RNeasy kit from Qiagen with on column DNase digestion.

Real-Time PCR. RNA was converted to cDNA using the IScript cDNA synthesis kit from Biorad. Fifteen μL of master mix consisting of 12.5μl of SYBR green supermix (BioRad), 1μL each of forward and reverse primer and 0.5 μL water were added to 10 ul cDNA and the IQ5 was programmed to run 40 cycles of 95°C for 30 sec., 60°C for 30 sec followed by melting curve analysis. Primer pairs were designed for androgen (AR), estrogen (ERα and ERβ) and progesterone (PR) receptors using PRIMER 3 software with GAPDH as an internal reference gene.

Statistical Analysis. Behavior expressed at 9 mo of age was summarized as investigatory and consummatory and analyzed using GLM methods of SAS (Ver. 9.1, Cary, NC). Effect of treatment was tested as the main effect with time and treatment by time interactions tested as subplot effects. Animal within treatment was used as the error term for treatment effects. Due to absence of normal data from rams at 18 mo of age, non parametric NPAR1WAY methods of SAS (Ver. 9.1, Proc Cary, NC) were used to analyze expression of AR, ER and PR receptors in brain tissues and concentration of serum testosterone . A paired T-test was used to determine treatment effects on behavior at 18 mo of age.

Results

Expression of investigatory behavior at the age of 9 mo was decreased (P = 0.03) in RU486 treated rams compared to control rams at the first exposure to estrous ewes (Fig.1), but not ($P \geq 0.4$) in subsequent tests. Consummatory behavior did not differ ($P \geq 0.24$) among treatment groups at any observation. By 18 mo of age, sexual behavior was not ($P \geq 0.4$) influenced by the treatment.

Serum concentrations of testosterone at 9 mo of age were greater (P = 0.06) in control rams than rams treated with RU486 (Fig. 2), but differences in serum concentrations of testosterone were not evident (P = 0.5) at 18 mo of age (Fig. 3).

Expression of mRNA levels for AR, PR and ERα did not differ at 18 mo of age among control and RU486 treated rams. Amygdala expression of ERβ, however, tended to be greater (P = 0.06) in RU486 treated rams than control rams (Fig. 4).

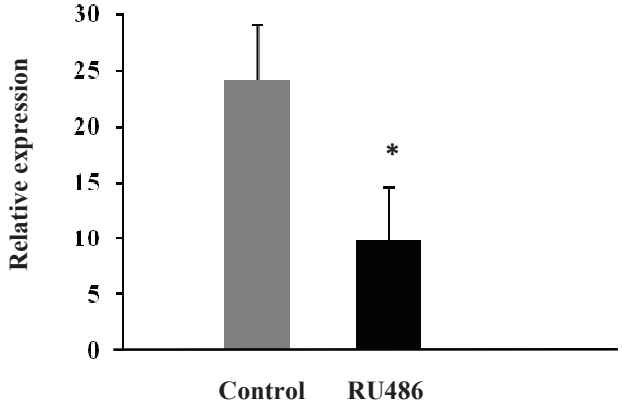

Figure 1. Relative expression of investigatory behavior at 9 mo of age in control and rams treated with RU486 from 4 to 8 wk *(P = 0.03).

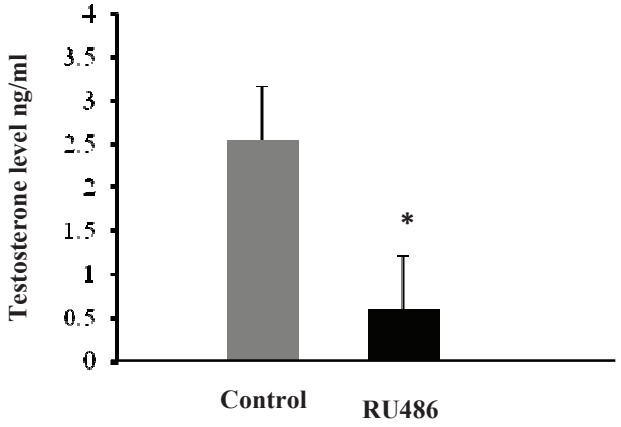

Figure 2. Serum concentration of testosterone (ng/mL) at 9 mo of age in control and ram lambs treated with RU486 from 4 to 8 wk *(P = 0.06) .

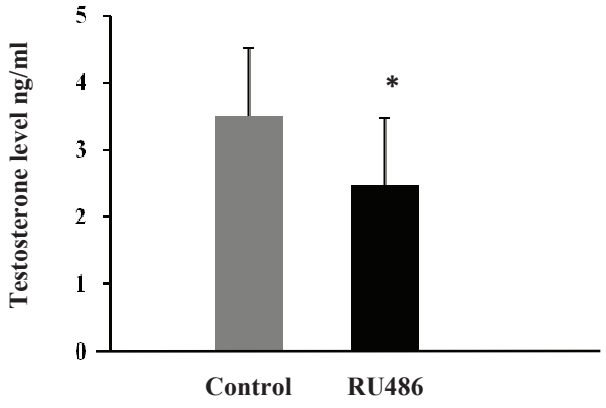

Figure 3. Serum concentration of testosterone (ng/mL) at 18 mo of age in control and ram lambs treated with RU486 from 4 to 8 wk *(P = 0.5).

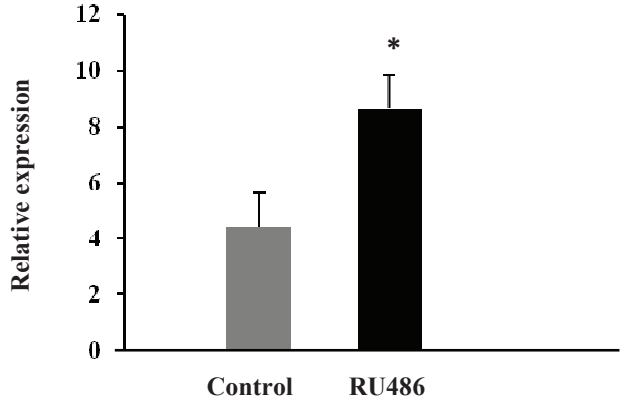

Figure 4. Relative expression of AMYG estrogen-β receptors at 18 mo of age in control and ram lambs treated with RU486* *(P* = 0.06).

Discussion

In the present study, RU486 treated rams tended to have increased amygdale expression of mRNA for ER-β in the amygdala than control rams. This increased expression of ER-β may be a result of alterations in ER-β during the postnatal period of sexual differentiation induced by exogenous RU486 treatment. The Role of the two major isoforms of estrogen receptor has been studied in the differentiation of mouse sexual behavior. Expression of ER-α is necessary for differentiation of normal reproductive function (Shughrue, et. al., 1997). Estrogen receptor–β is expressed in discrete nuclei of the hypothalamus and does not appear to be necessary for normal reproductive function (Hewitt and Korah, 2003). However, defeminization of male behavior in mice is influenced by expression of ER-β. Male mice lacking functional ER-β show increased expression of lordosis behavior than wild type males, but differences in the expression of male sexual behavior was not noted (Ogawa, et al., 1999; Kudwa, et. al., 2005).

In this study, testosterone production was reduced in RU486 treated male lambs at 9 mo of age but not at 18 mo of age. Treatment of RU486 may have delayed attainment of puberty in treated lambs through blocking the progesterone receptor in the testes. Although minimal amounts of testosterone are require to stimulate mounting behavior in sexually experienced males (Andersen and Tufik, 2006), a minimal threshold of testosterone may be required to initiate sexual behavior in sexually naïve males. Therefore the reduced production of testosterone in RU486 treated males may have inhibited initial sexual investigation of estrous ewes at 9 mo of age.

Conclusion

Blocking the PR during postnatal sexual development decreased sexual behavior during the initial exposure of rams to estrus ewes. Expression of subsequent behaviors may be a result of an incomplete inhibition of the PR during

development or increased positive stimuli provided by the rewarding aspects of sexual behavior.

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THE SELECTION VALUE OF PATERNITY INFORMATION ON CALVES DERIVED FROM MULTISIRE BREEDING PASTURES ON COMMERCIAL COW-CALF RANCHES

A.L. Van Eenennaam†*, K.L. Weber, K.L. Cooprider, L.J. Butler, D.J. Drake
†University of California, Davis, CA

ABSTRACT: The objective of this study was to examine the costs and benefits associated with deriving on-ranch weaning weight (**WW**) expected progeny differences (**rEPD**) using first calf crop performance data and DNA-based paternity designations. Paternity assignments for two consecutive calf crops were made for bulls present in multisire breeding pastures using a 99 SNP panel. The number of calves per sire varied from 0% to 96% of the calves in a pasture. Prolificacy in one year was not always predictive of prolificacy in the second year, even when sire groups remained unchanged. Calf WW data was used to calculate rEPD for 30 AI and ranch Angus sires. Because some bulls sired only a few offspring (range 0-26), the accuracy of their rEPD was correspondingly low. The American Angus Association (AAA) WW EPDs of the young herd bulls on this study averaged 17.6 kg (±2.1) and ranged in BIF accuracy from 0.05 to 0.25. The WW rEPD ranged from -11.0 to 10.0 kg (SD ±3.7) and BIF accuracies ranged from 0.03 to 0.42. The correlation between the AAA EPDs of well-proven AI sires and rEPDs was high (R^2 = 0.65), whereas the correlation between the low accuracy AAA EPD and rEPD for unproven ranch bulls was low (R^2 = 0.06). A simple economic analysis was performed by simulating the costs and benefits associated with the sale of a low WW rEPD terminal sire, based on his first calf crop progeny test by which time he would have sired a second calf crop as WW data are not available until after the breeding season, and his replacement with an average young sire for the remaining three breeding seasons. Assumptions included a) Bull: cost $2,500, 25 offspring per year, present for 5 breeding seasons, salvage value $900; and b) Calves: weaning sale weight 272 kg± bull′s on-ranch EPD at $2.27/kg, and genotyping cost of $20 per calf for the first year′s calf crop. It was estimated that culling the low rEPD bull at the completion of the progeny test would have a positive cost:benefit ratio if the WW rEPD was ≤ -16.7 kg. Selection based on WW rEPD would not have had a positive cost:benefit ratio for any of the 30 bulls on this study.

Key Words: on-ranch EPD, paternity testing, economic analysis

Introduction

Commercial herd bulls are a dead end from the standpoint of genetic evaluation. Although considerable variation exists in the genetic merit of young bulls with low accuracy expected progeny differences (**EPDs**), there is typically no way to improve this accuracy as breeding takes place in multisire breeding pastures to eliminate open cows due to sire failure. This precludes any evaluation of on-ranch progeny performance. DNA markers provide an approach to resolve the paternity of offspring produced in multisire breeding pastures, and this can be used to determine the genetic worth of herd bulls through on-ranch progeny testing (Van Eenennaam et al., 2007; Pollak, 2005).

A typical breed association EPD on young animals has a Beef Improvement Federation (**BIF**) accuracy of 0.05, although this is dependent upon the trait and whether an animal's own record is available. Paternity testing offers an approach to improve the accuracy of EPDs for any trait for which measurements are available through the development of on-ranch EPDs (**rEPD**), and possibly development of genetic estimates for traits which are not routinely measured by the seedstock sector. This approach has seen wide adoption in New Zealand where 20% of the ram breeding industry and 30% of the deer industry are now developing rEPDs based on DNA-based progeny assignments (McEwan, 2007). Perhaps most importantly, rEPDs offer an approach to identify which bulls are producing offspring that are best suited to the physical environment and commercial management conditions that are uniquely associated with each ranch operation.

There are data collection, animal identification, and genotyping costs associated with progeny testing and the development of rEPDs (Pollak, 2005). Provided selection decisions are not delayed to obtain progeny test results, genetic gain will be increased if the improvement in accuracy resulting from rEPDs enables better selection decisions. However, any change in management needs to be assessed from an economic perspective. Three studies have examined the economics of progeny testing on commercial cattle ranches. DeNise (1999) reported that the development of rEPDs from progeny testing was not profitable when considering post-weaning gain, whereas a simulation study found developing rEPDs for sale weight resulted in a positive return on investment under certain circumstances, such as the use of the same bulls in both a fall and a spring cow herd (Weaber, 2005). Likewise, it was concluded that there was a positive benefit-cost ratio when paternity information was used to cull 20% or more of the unproductive bulls (Gomez-Raya et al., 2008). These three papers used very different approaches and assumptions in their economic analyses.

Cow-calf producers cannot easily quantify the likely difference in future profit from using alternative bulls. A bull "selection by simulation" decision support tool (http://dss.ansci.iastate.edu; accessed 3/31/09) has been developed which predicts the expected phenotypic performance of offspring for a small portfolio of user-selected bulls (Garrick and Golden, 2008; Garrick, 2005). Phenotypes are then used to predict revenues and costs, including feed requirements, and present the user with an

estimate of the relative revenue and cost information associated with the use of each candidate bull. The objective of this study was to use this decision support tool, in conjunction with field data derived from a cow-calf operation, to examine the costs and benefits associated with deriving on-ranch weaning weight (**WW**) expected progeny differences on terminal sires using weaning weight data from a single calf crop, and DNA-based paternity designations.

Materials and Methods

Animal and ranch operation. The field study data was collected from the UC Davis commercial cow-calf herd housed at the Sierra Foothills Research and Extension Center, Browns Valley, California. Herd size is typically ~300 cows and cows calve during the fall months (September through November). Cows are bred in the winter months and all bulls passed a breeding soundness examination (**BSE**) by a licensed veterinarian prior to the breeding season. At calving, birth date, weight and dam ID are recorded, and a DNA sample is collected. At approximately seven months of age calves are weaned, weighed and a unique electronic identification device (**EID**) tag is assigned. Two to four herd bulls per breeding pasture were used and data was collected over a three year period 2006-2008. Weaning weight data was available on two of the calf crops. Some of the cows in this research herd were bred using A.I., and so the average number of open cows available per herd bull in this study was lower than is typically seen on Western ranches where a 25 cow:bull ratio is common. Bulls remained with the cows for a 90 day breeding season.

DNA samples were genotyped with 62 (2006), or 99 (2007, 2008) single nucleotide polymorphism (**SNP**) parentage panels (Igenity, Duluth, GA), and genotyping results were used to match potential sires to their true offspring. The calves sired by each bull in its breeding group was expressed as a proportion of that expected if all bulls in the pasture sired an equal number of progeny (e.g. expect 0.33 of the progeny to be sired by each bull if there were 3 bulls in the breeding group) for the three years of the study (Figure 1).

A genetic evaluation of 519 weaning weight records from two cohorts of progeny, sired by 23 herd bulls and 7 AI sires, born in Fall 2006 and 2007 was carried out using a maternal effects model of the form $y = Xb + Zu + Z_m u_m + Z_p u_p + e$, where y is a vector of weaning weight records adjusted by days of age at wean; X, Z, Z_m, and Z_p are incidence matrices relating observations to fixed effects, direct genetic effects, maternal genetic effects, and permanent environmental effects; b, u, u_m, and u_p are vectors of fixed effects, direct genetic effects, additive genetics effects on the dam of each individual, and nonadditive and permanent environmental effects due to the dam; and e is a vector of residuals. The fixed effects used in this model were year, sex and dam age (classified as 2, 3, 4, 5-10, and 11+ years of age). Heritabilities used were 0.25 and 0.10 for direct and maternal effects, respectively. The proportion of variance due to permanent environmental effects was assumed to be 0.05. Mixed model equations were solved using the MTDFREML package (http://aipl.arsusda.gov/curtvt/mtdfreml.html; accessed 3/31/09).

For the economic analysis, the cow-calf decision support software (http://dss.ansci.iastate.edu) was used to determine the value of one unit increase of WW EPD assuming a terminal sire breeding system (i.e. no replacement heifers kept). This was done by selecting the herd input values outlined in Table 1, and selecting the "Terminal Sire" breeding system. The cow genetics tab was set to "Zero EPDs" which sets all cow herd EPDs to zero. The status quo income, costs and capital value of the herd was then displayed by selecting the proceed button. To determine the value of selecting a bull with a one unit increase in weaning weight EPD, "Bogus" was entered as sire selection from the "Search Among" drop down menu, and then "Relative Economic Values" was selected from the "Type" drop down menu. The "Bogus" bulls are not real bulls, but represent information that can be useful to model the effect of changing the value of specific EPDs. Upon activating the "Select Bulls" button, a series of "bogus" bulls appeared. At this point the "REV WW" (relative economic value of WW) bull was selected (+1.0 WW EPD) along with the "REV Base" bull which is a bull with the value of 0 at all EPDs i.e. effectively the same genetics as cow herd. Selecting the "Calculate Perturbed Results" button resulted in an estimation of the added annual income resulting from use of the REV WW terminal sire. The value of one kilogram of WW EPD was valued at ~$42 per year.

In our simple model we considered the cost of DNA testing to be ~$500/bull (25 calves x $20/test) and of culling a bull based on a poor resultant WW rEPD, and then purchasing an average young sire to take his place for the 3 breeding seasons that the original bull would have been present in the herd. The breakeven in this scenario was when the loss in the weaning weight from the inferior bull for breeding seasons 3-5 equaled the cost of the genotyping his first calf crop and purchasing the new bull, minus the salvage value of the cull bull. We calculated that to be when the inferior bulls WW rEPD was $\leq$ -16.7 kg. If only steers were genotyped (~$250/bull), then this value would drop to $\leq$ -14.7kg.

Table 1. Production, management, and economic input values used for the simulated base herd

Model Parameters	Value
Herd size	300
Calving Rate	90%
Calf survival	95%
Mature weight	544 kg
Yearling weight	352 kg
Weaning weight	272 kg
Cows per bull	25
Maximum cow age, year	10
Incremental cow costs ($)	25
Heifer price ($)	2.11/ kg
Cow price ($)	1.11 kg
Calf price ($)	2.27/ kg
Bull price ($)	2,500
Years of service	5
Salvage Value of cull bull ($)	900

Results and Discussion

Calves were either assigned to a single sire (n=582), or were excluded from all sire candidates (n= 54). When using the 99 SNP panel in 2007/8, which has an exclusion probability (P_E) of 0.99999, over 95% of calves were assigned to a single sire. Large differences in calf output, ranging from 0 to 26 calves per bull per year, were observed (data not shown). Contrary to other studies (DeNise, 1999; Holroyd et al., 2002), there was no consistent trend in proportion of calves sired by a bull in multiple years (Figure 1). However, unlike those studies, the mix of bulls in breeding groups was rarely the same each year in this study, and that may explain the low repeatability of calf output observed in this data. The unpredictably small number of calves sired by some bulls was problematic from the perspective of developing accurate rEPDS. Additionally, these bulls were generating less revenue than was predicted.

Weaning weight rEPDs varied from -11.0 to 10.0 kg, whereas the AAA WW EPDs had a range of 13.1 kg for AI and natural service bulls, and only 6.3 kg for Angus herd bulls. The average accuracy of WW rEPD in this study was 0.24. Ironically, this is approximately the accuracy of breed association WW EPD when a bull's own weaning weight record is included in the WW EPD calculation.

According to the simple economic analysis presented in this paper, the development of WW rEPDs would not have been cost effective for any of the 30 bulls examined in this study. There are a number of additional considerations that were not addressed in this very simple analysis. Perhaps most importantly, the costs involved with genotyping bulls with average or superior WW rEPD were not addressed. There is no way to *a priori* select calves from only the potential culls. Genotyping a subsample of calves with extreme phenotypes (i.e. those with values in the tails of the normal distribution) could provide more information, (Goddard and Goddard, 1997), and be an approach to decrease the cost of genotyping (DeNise, 1999). However, this relies heavily on accurate adjustment factors for calf birth date and age of dam, and it might be difficult to make these adjustments on the fly at weaning. Genotyping progeny of only one sex may be a valid approach to decrease genotyping costs, although in this study it would have further decreased the accuracy of rEPDs from bulls with few progeny. The analysis also did not take into account the fact that the young replacement bull purchased would be available for an additional two years of service in the herd. The economic analysis would be different in self-replacing herds because less offspring would be harvested each year, and the genetic merit of the cow herd would increase annually. As a result, the harvested offspring would exhibit twice the rate of improvement that would occur in a terminal sire system.

Implications

The costs involved in developing rEPDs on commercial ranches must be outweighed by a resultant increase in performance and revenues. It seems likely that deriving value from additional phenotyping for economically-relevant traits (e.g. heifer pregnancy) for which breed-based EPDs are currently unavailable would be required to make the development of rEPDs cost-effective for genetic improvement on commercial cow-calf ranches. The decision support software used in this analysis is a useful tool to predict the difference in future profit associated with alternative bull selection decisions.

Acknowledgements

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Figure 1. Sire prolificacy of herd bulls with calves in more than one year expressed as a proportion of expected (1.0; dashed line) based on the number of bulls present in multisire breeding pastures. Parentage was determined by SNP analysis of DNA from progeny and sires. All bulls were Angus except for the two marked with an asterisk (*) which were Horned Herefords.

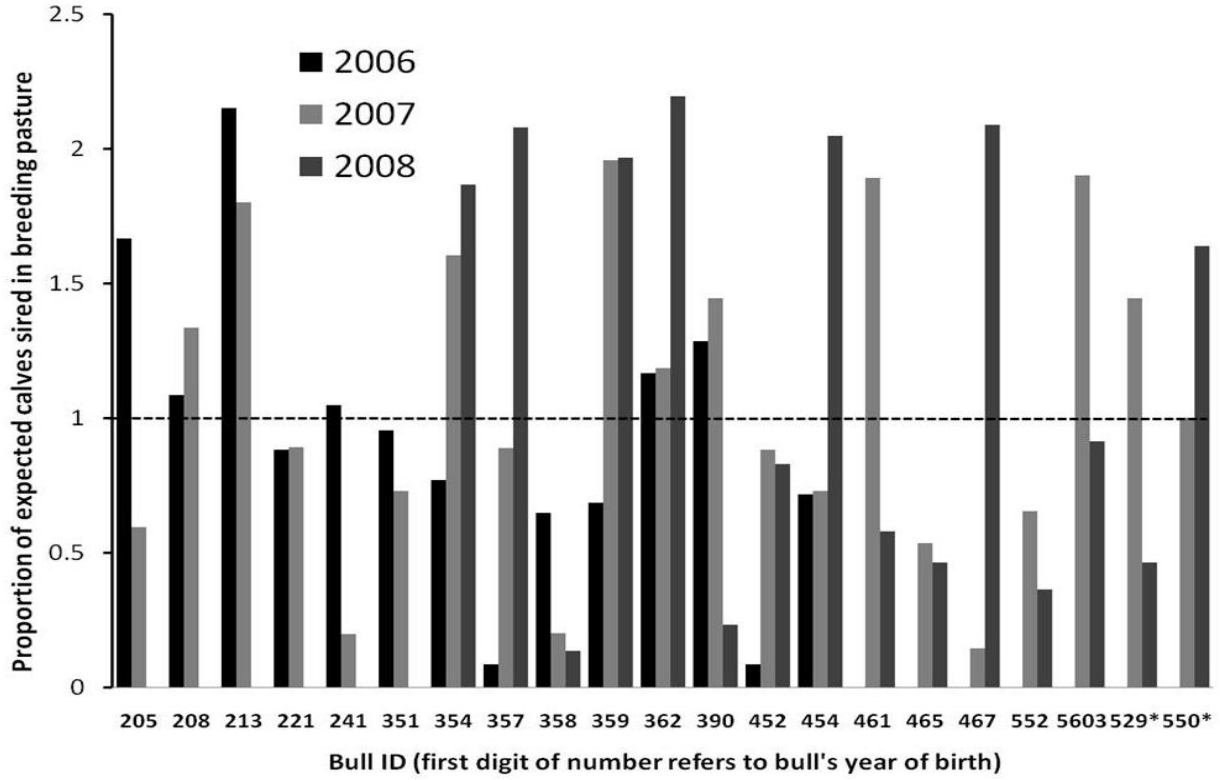
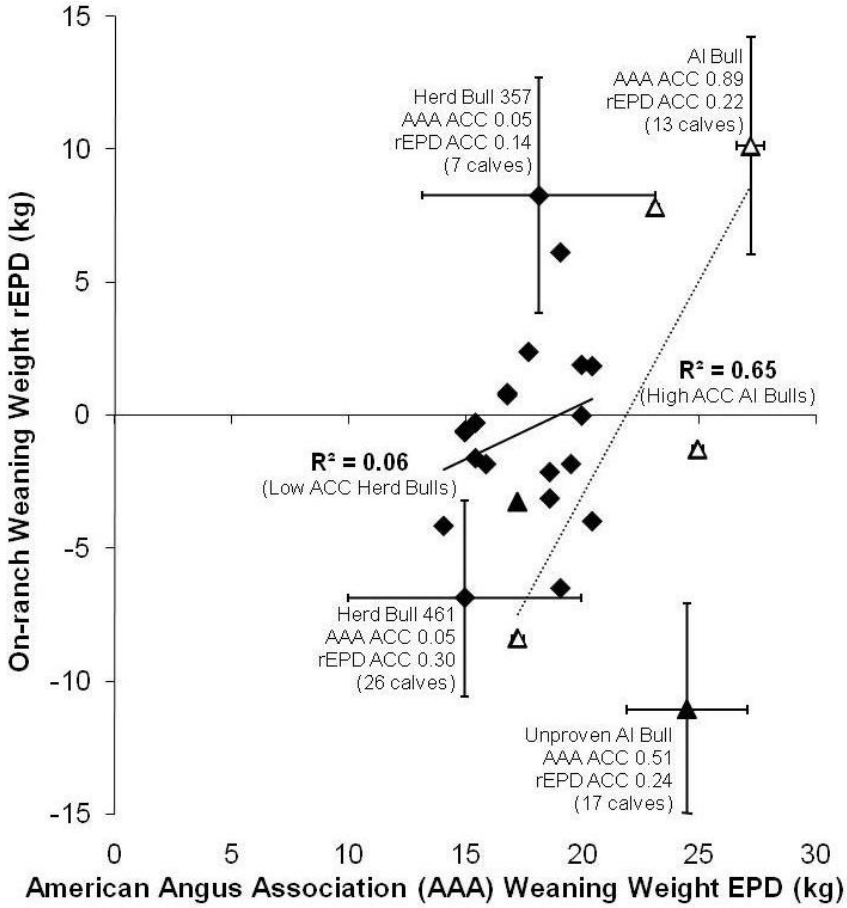


Figure 2. Comparison of American Angus Association (AAA) weaning weight (WW) EPDs and on-ranch EPDs (rEPD) for 20 Angus herd bulls (♦) and 4 high-accuracy (AAA ACC 0.89-0.97) AI sires (Δ) and two unproven (AAA ACC 0.4-0.51) AI sires (▲). Confidence intervals (67%) based on BIF accuracies are plotted for the highest and lowest WW rEPD herd and AI bulls. The American Angus Association (AAA) WW EPDs of the young herd bulls on this study averaged 17.6 kg (±2.1) and ranged in BIF accuracy from 0.05 to 0.25. The WW rEPD ranged from -11.0 to 10.0 kg (SD ±3.7) and BIF accuracies ranged from 0.03 to 0.42 for bulls with 1 and 40 calves, respectively. Because some bulls sired only a few offspring in a breeding season (range 0-26), the accuracy of their rEPD was correspondingly low. The correlation between AAA WW EPDs and rEPDs was much higher for the well-proven AI bulls (R^2=0.65, dashed line) than for the low-accuracy herd bulls (R^2=0.06, solid line). The AAA WW EPD of the all the herd bulls fell within a single 67% confidence interval based on the lowest AAA WW EPD ACC of 0.05.



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GROWTH ENDOCRINE AXIS AND BOVINE CHROMOSOME 5: ASSOCIATION OF SNP GENOTYPES AND REPRODUCTIVE PHENOTYPES IN AN ANGUS, BRAHMAN AND ROMOSINUANO DIALLELE

P. Luna-Nevarez[1], G. Rincon[2], D.G. Riley[3], C.C. Chase, Jr.[3], J.F. Medrano[2], D. VanLeeuwen[1], G.A. Silver[1], and M.G. Thomas[1]

[1]New Mexico State University, Las Cruces, New Mexico 88003
[2]University of California, Davis, California 95616
[3]USDA-ARS, Subtropical Agricultural Research Station, Brooksville, Florida 34601

ABSTRACT: The growth endocrine axis influences reproduction. A QTL associated with enhanced ovulation exists on chromosome 5 in cattle and there are 6 genes underlying this region involved in the mechanisms of GH action. Resequencing exons, 5' and 3' untranslated regions and conserved non-coding regions of these genes in a multi-breed resource population revealed 75 SNP usable for genotype to phenotype association studies. In the current study, phenotypes included age at first calving, calving interval, days to calving, and pregnancy rate. Data were collected from developing heifers (n = 650) of a diallele composed of Angus, Brahman, and Romosinuano breeds. A SNP in the promoter of the signal transducer and activator of transcription (STAT)2 gene, which is a second messenger of GH, had minor allele frequency > 10% across the three breeds. This SNP did not deviate from Hardy-Weinberg equilibrium (X^2 = 1.00, P > 0.31), so deemed useful for genotype to phenotype association analyses. Since the remaining SNP appeared to predict breed, they were used to correct for population stratification using STRUCTURE, which revealed three distinctive ancestral clusters. No significant association was detected between the STAT2 genotype and reproductive traits in mixed effects analyses using genotype as a fixed term, sire as a random term, and coefficient of ancestry as a covariate; however, the interaction of SNP genotype and ancestral cluster was associated with the traits days to calving (P < 0.05) and calving interval (P < 0.10). Interaction plots revealed a higher estimated effect of heterozygous genotype in cluster 1 (inferred primarily from Brahman) and lower estimates in clusters 2 and 3 (inferred primarily from *Bos taurus*). The heterozygous genotype extended these trait levels ~100 d. A SNP in the promoter of the STAT2 gene was associated with fertility trait levels in admixed cows of the breeds Angus, Brahman, and Romosinuano. The effect appeared to be a non-additive genetic relationship as heterozygous genotype extended levels of traits indicative of postpartum rebreeding.

Key Words: heifer, reproduction, SNP genotype.

Introduction

Components of the GH/IGF axis play important roles in reproductive processes, such as folliculogenesis, steroidogenesis, and embryonic development. Growth hormone acts through its receptor (GHR) to increase hepatic secretion of IGF-1 as a consequence of activating cell signaling pathway proteins (Lucy, 2008).

A QTL associated with ovulation and (or) twinning in cattle exists on chromosome 5, and the IGF-1 gene underlies this region (Allan et al., 2009; Kim et al., 2009). There were other genes underlying this QTL known to be involved in the GH/IGF axis, suggesting these genes may also be regulators of fertility.

Brahman cattle are typically slower to reach sexual maturity compared to most *Bos taurus* breeds (Lopez et al., 2006). Objective herein was to evaluate associations of genotypes from SNP derived from candidate genes of the GH/IGF axis with reproductive phenotypes of beef heifers from the breeds Angus, Brahman and Romosinuano.

Materials and Methods

Genes and SNP Discovery

Genes in the GH/IGF-1 endocrine axis from a 23 Mb QTL-region of bovine chromosome 5 were identified on the GenMAPP diagram of Farber et al. (2006). These genes were IGF-1, IGFBP6, Pro-Melanin Concentrating Hormone (PMCH), Signal Transducers and Activators of Transcription 2 and 6 (STAT2,6) and Suppressor of Cytokine Signaling 2 (SOCS2). Functional regions of each gene were resequenced using DNA from 48 unrelated cattle according to the procedures described by Rincon et al. (2007) and Garrett et al. (2008). They were: 1000 bp of the 5'-untranslated region, exons, and the 500 bp of the 3'-untranslated region. If a gene contained multiple exons (> 10), then a Vista alignment (http://pipeline.lbl.gov/) was conducted to identify conserved introns and exons. Resequencing was completed at SeqWright (Houston, TX) and provided results of SNP, indel, and microsatellite. Finally, SNP were confirmed with CodonCode aligner (CodonCode® Corporation, Dedham, MA) and then tested to be synonymous or non-synonymous using Polyphen (http://genetics.bwh.harvard.edu/pph), and if they were a tag SNP (Haploview; http://www.broad.mit.edu/mpg).

Animals, Phenotype, and Genotype

Angus, Brahman, and Romosinuano heifers and their reciprocal crosses from a diallele design (n = 650) were born from 2002 to 2005. These heifers were raised in the cow-calf system of the USDA-ARS, Subtropical Agricultural Research Station, in Brooksville FL.

After weaning, heifers were exposed to bulls until determined to be pregnant by rectal palpation. After calving, heifers were exposed to bulls for ~90 d from March 20 to June 20 each year. First calving date was collected and reported as the trait, age at first calving. Second calving date was collected and used to calculate, calving interval, as the difference in days between first and second calving. Similarly, the trait, days to calving, was calculated as the difference in days between the second calving date and the first date of the last breeding season that heifers were exposed to bulls. The categorical trait, pregnancy rate, was determined after the second breeding season.

Genotyping was performed using 25 ng/μL of DNA from each heifer. Amplicons derived from PCR and the Sequenom MassArray platform were used to determine SNP genotypes (GeneSeek, Inc., Lincoln, NE). Genotypes were coded 11 for homozygous, 12 for heterozygous, and 22 for opposing homozygous.

Statistics

Analyses were conducted using SAS (Version 9.2; SAS Inst. Inc., Cary, NC), which included Genetic Analysis Tools of SAS (Saxton, 2004).

Simple Statistics and Frequencies

Simple descriptive statistics for growth traits (e.g., birth weight, 205-d weight, and 365-d weight), and continuous reproductive traits (e.g., age at first calving, calving interval, and days to calving), were calculated using PROC MEANS. Pregnancy rate was calculated using PROC FREQ. Allele and genotype frequencies, as well as deviation from Hardy-Weinberg equilibrium, were estimated with PROC ALLELE.

Population Stratification Analyses

Because an admixed population was involved in this study, STRUCTURE was used to infer population stratification (Pritchard et al., 2007). This program estimated each heifer's proportion of membership or admixture termed, Ancestry Coefficient.

Association of Genotype to Phenotype

Association analyses were conducted using PROC MIXED for continuous traits and PROC GLIMMIX for categorical traits. Only polymorphisms with genotype frequencies greater than 10% were considered appropriate to be included in association analyses. The genotype to phenotype association model was:

$y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + E_m + F_n + e_{ijklmn}$, where

y_{ijklmn} = phenotypic value of trait,
μ = population mean,
A_i = fixed effect of SNP genotype,
B_j = fixed effect of year of birth (i.e., 2002, 2003, 2004, and 2005),
C_k = fixed effect of age of dam (i.e., 2, 3, 4, 5 to 10, or 11 yr and older; BIF, 2006);
D_l = covariate of coefficient of ancestry (i.e., admixture proportion from inferred Brahman cluster),
E_m = covariate of ordinal birth date of the heifer,
F_n= random effect of sire using the *Z* statistic to test if Ho:$\sigma_w^2 = 0$, and
e_{ijklmn} = random residual error.

The association model was also executed with either breed or cluster category and their interaction with genotype as a fixed effect, which replaced the covariate, coefficient of ancestry. Proc GPLOT was used to visualize the interaction.

If genotype term was found to be a significant ($P < 0.05$) source of variation in association analyses for continuous traits, preplanned pairwise comparisons of least squares means were generated with PDIFF. These mean separation tests were executed within LSMEANS in the mixed procedure, which included Bonferroni adjustment.

Results and Discussion

Results of resequencing and related bioinformatics revealed 75 SNP in the genes of GHR, IGF-1, IGFBP6, PMCH, SOCS2, STAT2, and STAT6 (Rincon et al., 2007; Garrett et al., 2008). Only a SNP in the promoter of the STAT2 gene had minor allele frequency > 10% across the three breeds. This SNP did not deviate from Hardy-Weinberg equilibrium ($X^2 = 1.00$, $P > 0.31$). Population stratification analysis was executed with 56 of the SNP useful as ancestral informative markers (i.e., minor allele frequency < 10%), which suggested 3 clusters of ancestral subpopulations in this study (Table 1). Genetic markers are commonly used to correct for population stratification using structured methods (Pritchard et al., 2007). In this study, the admixed population included one *Bos indicus* breed (Brahman), two *Bos taurus* breeds (Angus and Romosinuano), and their reciprocal crosses. The genetic structure observed herein suggested Brahman cattle were a unique breed-group because genetic structure arose primarily from one cluster. Structure output data from Angus and Romosinuano breeds revealed that they shared historic ancestry. Similar results were reported by McKay et al. (2007) using 2 *Bos indicus* and 8 *Bos taurus* breeds.

Table 2 includes the simple statistics of the traits evaluated in this study. Year of birth ($P < 0.01$), coefficient of ancestry ($P < 0.05$) and sire ($P < 0.05$) were significant sources of variation in prediction of reproductive traits. No significant association was detected between the STAT2 SNP genotypes with any reproductive trait using the model that included coefficient of ancestry as covariate. However, these genotypes interacted with cluster for prediction of the traits days to calving ($P < 0.05$) and calving interval ($P <$

0.10). Mixed model analyses of these traits involving the interaction of cluster and genotype revealed a higher estimated effect of heterozygous genotype (~100 d) in cluster 1 (inferred from Brahman), and lower estimates in clusters 2 and 3 (inferred from *Bos taurus* breeds). Sliced interaction plotting (Figure 1) also revealed that the heterozygous genotype appeared unfavorable, which suggested a non-additive genetic effect. This plot was only of the purebred cattle.

The STAT proteins are involved as signal transducers of interferon actions on uterine epithelium during early pregnancy, including uterine receptivity and maternal immune response (Schindler and Plumlee, 2008). The STAT proteins are also important intracellular second messengers of GH, IGF, and leptin, which are involved in puberty and postpartum rebreeding (Fruhbeck, 2006). In the current study, a SNP in the promoter of the STAT2 gene was found to be associated with the traits days to calving and calving interval. The interaction among STAT2 genotypes for these traits and ancestral cluster suggested a non-additive relationship as the heterozygous genotype appeared to extend calving interval and days to calving.

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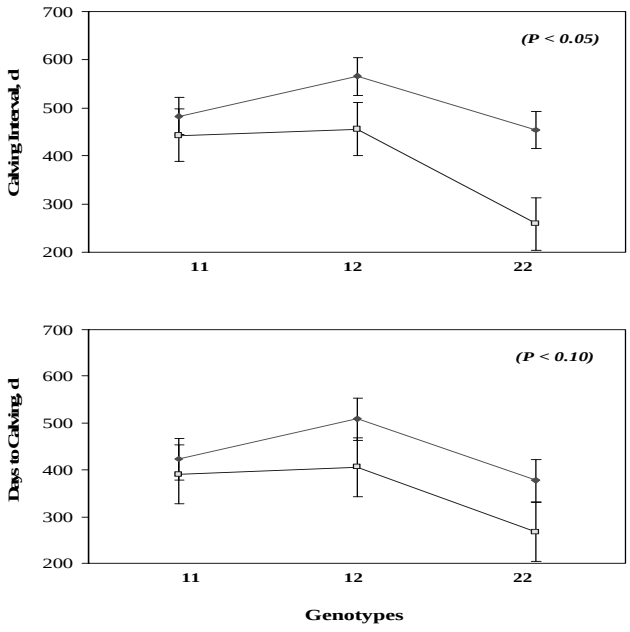

Figure 1. Graphic representation of the interaction among SNP genotypes in STAT2 (e.g., 11, 12 and 22) and ancestral clusters for the traits calving interval and days to calving. Clusters from *Bos indicus* (1; upper line) and *Bos taurus* (2 and 3; lower line) purebreds are represented.

Table 1. Ancestry proportions of Angus, Brahman and Romosinuano diallele breed groups in clusters outputted from STRUCTURE.

		Ancestry Clusters		
Breed Group	N	1	2	3
Angus	66	0.012	0.694	0.294
Brahman	59	0.878	0.029	0.082
Romosinuano	129	0.040	0.461	0.498
Angus x Brahman	116	0.462	0.334	0.204
Angus x Romosinuano	136	0.039	0.497	0.464
Brahman x Romosinuano	143	0.474	0.234	0.292

Table 2. Number of observations, and mean ± SE for birth weight, 205-d weight, 365-d weight, age at first calving, calving interval, days to calving, and pregnancy rate in a diallele of Angus, Brahman and Romosinuano heifers.

Breed Group	N	Growth Traits[1]			Reproductive Traits			
		Birth Wt (kg)	205-d Wt (kg)	365-d Wt (kg)	Age at First Calving (d)	Calving Interval (d)	Days to Calving (d)	Pregnancy Rate (%)
Angus	66	29.1±0.4	171.9±2.1	237.9±4.0	796.4±18.7	465.9±24.6	405.8±26.0	84.8
Brahman	59	30.2±0.5	200.2±2.3	252.2±2.9	934.9±27.7	546.4±24.9	470.1±26.1	69.4
Romosinuano	129	29.7±0.2	176.7±1.8	222.4±2.5	774.4±7.2	433.3±13.8	389.8±15.4	89.9
Angus x Brahman	116	33.1±0.3	208.1±2.4	281.0±2.7	763.8±13.0	421.4±11.1	357.0±11.6	91.3
Angus x Romosinuano	136	30.8±0.3	187.3±1.8	249.2±2.2	721.6±8.9	450.7±12.2	364.9±11.5	88.9
Brahman x Romosinuano	143	33.0±0.4	207.0±1.8	265.2±2.3	786.1±9.3	423.0±12.2	372.4±12.9	90.2

[1]Traits adjusted according to Beef Improvement Federation guidelines (2006).

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AGGREGATE STAYABILITY – USING INFORMATION FROM YOUNGER AGES

B. W. Brigham[1], S.E. Speidel[1], D. J. Garrick[2], L.D. Keenan[3], and R. M. Enns[1]

[1]Department of Animal Science, Colorado State University, Fort Collins, CO 80523
[2]Department of Animal Science, Iowa State University, Ames, IA 50011
[3]Red Angus Association of America, Denton, TX 76207

ABSTRACT: Stayability reflects productive life which has direct impacts on profitability of cow-calf operators in the beef industry. The traditional definition of stayability EPD is the probability a cow will remain in production through 6 yrs of age. Its implementation has been problematic for producers with the most common concern that a female does not get an observation until she has reached the 6yr benchmark resulting in low accuracy EPD for animals until late in life. The objective of this study was to estimate genetic correlations between traditional stayability and stayability to younger ages in order to use earlier observations to improve the prediction of EPD for stayability to 6yr. Data were from the Red Angus Association of America. Ages considered as earlier indicators of stayability to 6yrs of age were stayability to 3yrs, 4yrs and 5yrs. Variance components were estimated with a sire model using a probit threshold analysis including contemporary group as a fixed effect. Heritabilities calculated at each age were, 0.11, 0.12, 0.11 and 0.12 for 3, 4, 5, and 6yr stayability respectively. Genetic correlations were calculated using Gibbs sampling techniques. The genetic correlations among pairs of age definitions were 0.84, 0.46, 0.49 for 3yr-4yr, 3yr-5yr, 3yr-6yr, 0.85, 0.70, for 4yr-5yr, 4yr-6yr, and 0.60 for 5yr-6yr. Data used in the subsequent EPD calculation was structured to be independent at each age definition to avoid covariances between residuals. Aggregate stayability values were calculated using the genetic correlations among ages to weight information from each age definition. Very little re-ranking among sires with accuracy greater than 0.60 occurred, resulting in a rank correlation of 0.99 between conventional 6yr stayability EPD and aggregate 6yr stayability EPD. An average accuracy increase of 0.07 was found in young animals that had yet to have daughters reach the 6yr benchmark age. Publishing stayability computed in this manner will help to eliminate issues associated with low accuracy EPD for animals younger the 6yrs of age.

Key words: beef cattle, index, longevity, stayability

Introduction

Sires' daughter fertility and stayability are both very important aspects to consider when making bull selection decisions. Stayability benefits a herd's productivity in several ways including decreasing the need and cost for young replacements and thereby resulting in higher average weaning weights because of the greater average weaning weight of calves from older cows (Garrick, 2006). In beef cattle the only genetic prediction available to assist producers in selection for long term reproductive ability is stayability, which is defined as the probability a cow will remain in production to 6 yrs of age given she calves as a 2 yr old. In a herd where only 60% of the cows remain until 6 years of age, a one unit increase in overall herd stayability EPD has been valued at nearly $2000.00 (Enns et al., 2005).

Research supports that a high proportion of females in production at 4 yr will still be in production to 6 yr or more, suggesting that earlier measures could be used as indicator traits in the genetic prediction of stayability to 6 yr of age. Martinez et al., (2005) reported correlations between 4 and 5 yr or 4 and 6 yr stayability of 0.85 and 0.86, respectively. Anecdotally, cattle producers support the premise that a female conceiving at 4 yr of age will remain in the herd until 6 yr of age. Cows culled after conceiving as a 4 yr old are most likely culled for reasons unrelated to reproductive ability.

A difficulty typical of any long term prediction of female fertility is the requirement for a benchmark age. In the case of stayability it takes 6 yrs to receive a stayability observation. At that age, the sire of that female will be at least 8 yr old resulting in very low prediction accuracy for sires less than 8 yr of age. Most sires are no longer active at 8 yr of age unless available through artificial insemination. This lag between accurate prediction of stayability and the need for young replacement sires has received some criticism in the literature (e.g., Hudson and Van Vleck, 1981). As a result, the objective of this study was to estimate the genetic correlations among earlier stayability ages and use this earlier age information in an aggregate 6yr stayability genetic prediction.

Materials and Methods

Data used in this analysis were obtained from an existing database and not subject to Animal Care and Use Committee approval.

Herd book data from 1944 to 2007 was supplied by the Red Angus Association of America (RAAA). Research was conducted in two phases; first heritability estimates among the alternate stayability endpoints were

calculated. Secondly EPD for each age endpoint and genetic correlations among the endpoints were calculated and subsequently combined into a single aggregate stayability breeding value. Data preparation and editing for each phase will be described separately.

Variance component estimation

Variance components were estimated for each stayability benchmark, 3 yrs, 4 yrs, 5 yrs and 6 yrs of age. Stayability observations were assigned to dams based on their age in days at each calving. In order for a dam to be eligible to receive a stayability observation for a given endpoint, she was required to be at least as old as the defined benchmark. Dams were required to calve on an annual basis within a 60d time period of their previous calving. Dams meeting these criteria received a favorable designation (1) while dams who had not met the requirements received un-favorable score (0). A 3 generation sire pedigree was constructed for each age definition, building recursively from animals with observations.

Contemporary groups were formed based upon the breeder (owner) code of the dam and the breeder (owner) code of each calf born. In order to be placed in a contemporary group a dam must have a breeder code as well as a breeder code of a calf meeting the defined stayability definition. For purpose of variance component estimation, data volume was further reduced by restricting contemporary group size to a minimum of 5 individuals and only groups with variation were included. Contemporary group was included as the sole fixed effect.

Variance component estimation was performed using ASREML (Gilmor et al., 2002) fitting residual maximum likelihood linear sire models with the PROBIT option for categorical data.

Breeding value and genetic correlation estimation

The data used for the estimation of breeding values was the same used for the previously described variance component estimation. Categories of stayability definitions and criteria to receive an observation were also the same as previously described. Independent data sets were formed for each stayability age, meaning animals that were eligible for an older stayability definition were not considered in younger categories. Consequently stayability to ages 3 yr, 4 yr, and 5 yr were limited to single birth year groups. A three generation pedigree including animals with observations from all age definitions was constructed and this single pedigree was used in each analysis.

Contemporary group formation was the same as previously described with the exception of not being sifted for within group size. Single animal contemporary groups were removed from the data.

Model:

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

$$Y = X\beta + Zu + e$$

where

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

In the above set of equations, Y is equal to a vector of transformed observations on the underlying scale, X a known design matrix relating fixed effects to those individuals in vector Y. In this case the only fixed effect included was stayability contemporary group contained in vector β. Z was a design matrix relating the random additive genetic effects in *u* to the individuals in vector Y, and e a vector of random residual errors. A is Wright's additive numerator relationship matrix, I is an identity matrix with an order equal to the number of observations in Y. σ_a^2 and σ_e^2 were additive and residual variances, respectively. The additive genetic variance (σ_a^2) was specific to each stayability age definition for each set of EPD calculated. In accordance with MAP models the residual variance (σ_e^2) is forced to be equal to 1.

Genetic correlations among each stayability age definition were calculated using the THRGIBBSF90 program from the package BLUPF90 described by Misztal et al. (2002). Pairwise combinations of stayability age definitions were fitted in a bivariate threshold model using the same fixed effects as the model for variance component estimation. A burn in period and sampling period of 10,000 and 50,000 respectively were used to estimate correlations for all pairs.

The aggregate stayability EPD was calculated by weighting each individual observation with the genetic covariance among the age definitions using the equation presented below.

$$Y = \left[diag\left[Z'RZ\right] + G_0^{-1}\right]u$$

In the above equation, Y is a vector of stayability observations within a given animal on the underlying scale, Z'RZ is a trait within animal accumulation of diagonal elements from the random portion of the coefficient matrix. Individual animals are represented by square matrix with an order equal to number of traits by number of traits. G_0 is the variance/covariance matrix for all age definitions and *u* is a vector of aggregate breeding values.

Results and Discussion

Stayability was first published by Snelling et al. (1995) where beef cow stayability was shown to be a trait moderate in heritability and economically important to cow-calf producers. This initial study was conducted using two cow-calf operations of different management and environmental conditions. Both of the herds used in the study had a phenotypic stayability of 38% of cows' achieving the 6 yr benchmark. Heritabilites were calculated

to be 0.11 and 0.14 in the two herds. The study included four ages, 3, 6, 9, 12 years. The definition of 6 years of age was selected being identified as the break even age for a cow to pay for her own replacement costs as well as for those cows not making the benchmark as well as the heritability was comparable to the other ages investigated. In this study, earlier age of 3 yrs, 4yrs, 5yrs as well as the traditional 6 yrs were considered in an effort to improve accuracy of EPD at earlier ages.

Summary statistics for each phase of the project are given in table 1. Data counts of favorable and un-favorable observations are given for each age benchmark as well as pedigree size.

Table 1. Summary of observations and pedigree size for each stayability age definition by analysis

Stayability Age	Observation	Variance Component data count	EPD data count
3 yr	Yes (1)	155,352	8,509
	No (0)	80,125	6,211
	Pedigree	58,193[1]	381,346[2]
4yr	Yes (1)	124,892	7,024
	No (0)	124,053	7,995
	Pedigree	58,006[1]	381,346[2]
5yr	Yes (1)	94,509	5,410
	No (0)	138,216	9,473
	Pedigree	53,661[1]	381,346[2]
6yr	Yes (1)	53,327	22,073
	No (0)	132,120	52,915
	Pedigree	43,797[1]	381,346[2]

[1]Sire model pedigree
[2]Animal model pedigree

Heritability and genetic correlations for each age definition are shown in table 2. In general heritabilies were similar to previous research (Snelling et al., 1995, Martinez et al., 2005) ranging from 0.11, 0.12, 0.11 and 0.12 at 3yr, 4yr, 5yr, and 6yr stayability respectively. Genetic correlations among age definition pairs were moderate to high. Adjacent stayability ages tended to be the higher genetic correlations as would be expected since less time has passed between these traits. Martinez et al. (2004) found similar genetic correlations in a population of Hereford cows ranging from 0.75 to 0.90.

Table 2. Estimates of heritability and genetic correlation for each stayability age definition.

Stayability Age Definition	3yr	4 yr	5 yr	6 yr
3yr	0.11 (0.0009)	0.84	0.46	0.49
4yr		0.12 (0.0009)	0.85	0.70
5yr			0.11 (0.0009)	0.60
6yr				0.12 (0.01)

Heritabilites and standard error () on the diagonal and genetic correlations above the diagonal.

EPD from traditionally defined stayability, using only the 6 yr benchmark, was comp aired to aggregate stayability EPD, using information from all previous ages. Average EPD and genetic trends were very similar between the methods. Rank correlations between traditional 6yr stayability and aggregate 6yr stayability are presented in table 3. Comparing the traditional 6 yr stayability EPD versus aggregate 6 yr stayability EPD the rank correlations were high, ranging from 0.89 to 0.99. Sires with accuracy greater than 0.70 had a rank correlation of 0.99, demonstrating that for well proven animals indexing all stayability age information does not have a large impact on EPD. Active females showed the greatest amount of re-ranking between these two methods resulting in a 0.89 rank correlation, a result of the additional information added when all stayability ages are considered.

Table 3. Rank correlations of traditional 6yr stayability EPD and 6yr aggregate stayability

Animal group	n	Rank Correlation
All Animals	380,246	0.96
Sires accuracy 0.70 or greater	177	0.99
Active Dams	22,845	0.89
Non-Parents	124,980	0.95

Combining the individual EPD information into an aggregate EPD showed an increase in accuracy for all animals. Figure 1 plots the average BIF accuracy by birth year of all animals. The accuracy increase relates to the additional information being added to these predictions from the younger age definitions of stayability. Animals representing the 3 yr, 4 yr and 5 yr stayability observations corresponded to 2004, 2003 and 2002 birth years. These years in particular are those which would not receive observations if younger ages were not considered. The average accuracy of only these animals is shown in figure

2. The average accuracy increase of 0.07 was observed among these groups. Although quantitatively this increase does not seem great, it represents a doubling of the accuracy for these low accuracy animals.

Implications

The ability to predict stayability of a bull's daughters accurately earlier would be beneficial to cow-calf producers both financially and managerially. These results show earlier ages are in the same range of what is currently used in national genetic evaluations. This method to combine all available data on females will be of greatest benefit to young sire selection.

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Figure 1. Average stayability EPD accuracy by birth year of all animals.

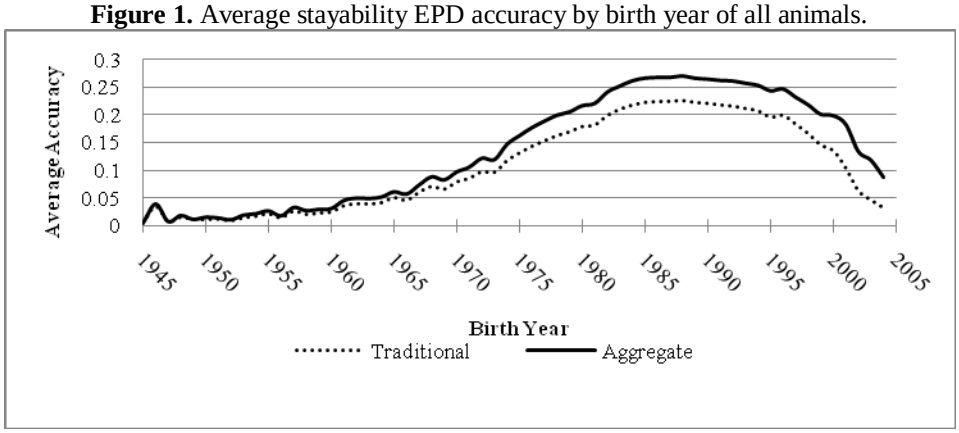
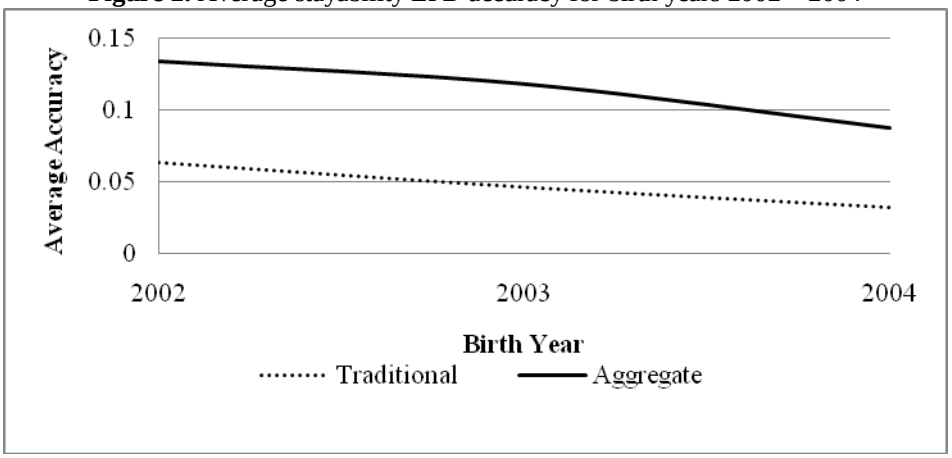


Figure 2. Average stayability EPD accuracy for birth years 2002 – 2004



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PHENOTYPIC RELATIONSHIPS OF RESIDUAL FEED INTAKE WITH GROWTH AND CARCASS PERFORMANCE TRAITS OF SPRING-BORN ANGUS BULLS[1]

G.E. Berryhill[2], S.P. Doyle[2], and L. Rechel[3]
[2]College of Agriculture, California State University, Chico, CA 95929
[3]Snyder Livestock Company, Inc., Yerington, NV 89477

ABSTRACT: With rising feed costs, increasing feed efficiency through reducing feed inputs is imperative. Thus, the phenotypic relationships of residual feed intake with growth and carcass performance traits were determined in spring-born Angus bulls at a central bull test station in Yerington, Nevada. Individual feed intake data were collected for bulls (n=136) using the GrowSafe feeding system. Residual feed intake (RFI) was calculated as the difference between actual intake and intake predicted by the stepwise linear regression of DMI on ADG, mid-test metabolic body weight (MMWT), and ultrasound back fat (uFT). Based upon calculated RFI, animals were divided into low (≤–0.5 SD; n=35; RFI=–1.036 kg/d), marginal (±0.5 SD; n=60; RFI=–0.001 kg/d), and high (≥0.5 SD; n=41; RFI=0.886 kg/d) groups. Group means were analyzed by ANOVA, fitting RFI grouping as the independent variable. Pearson correlations were determined for RFI and ADG, MMWT, feed conversion ratio (DMI/ADG; FCR), partial efficiency of growth (ADG/DMI for growth; PEG), ultrasound ribeye area (uREA), uFT, ultrasound percent intramuscular fat (uPIMF), uREA EPD, uFT EPD, and uPIMF EPD. No significant differences existed among RFI groups for on-test weight (P=0.70), off-test weight (P=0.48), ADG (P=0.58), uREA (P=0.12), uPIMF (P=0.82), and uFT (P=0.44). Furthermore, no significant group differences were detected in uFT, uREA, or uPIMF EPDs (P≥0.05). Significant group differences were detected in FCR, PEG, DMI, and RFI (P=0.00). Low RFI grouped animals appeared to exhibit favorable FCR (5.74 kg) and PEG (0.49) in conjunction with reduced DMI (11.12 kg/d). RFI was significantly correlated (P=0.00) with FCR, DMI, and PEG (0.49, 0.70, –0.89, respectively), further supporting the group analysis. Results suggest that phenotypic selection for decreased RFI can be used to improve feed efficiency without adversely impacting carcass composition and growth performance.

Key Words: Beef Cattle, Residual Feed Intake, Carcass Trait

Introduction

Optimizing feed efficiency is of critical importance to a livestock operation as feed costs account approximately 60% of production costs in commercial beef operations (Arthur et al., 2001). Thus, it is imperative that producers have at their disposal appropriate tools to make selection decisions to optimize outputs while minimizing inputs.

Several measures of feed efficiency have been employed as selection tools, yet they are not without adverse effects. Traditionally, emphasis has been placed on growth rate and FCR to achieve greater efficiency in production, yet selection for growth rate has resulted in an increase in mature cowherd size, requiring greater resources for cowherd maintenance (Herd and Bishop, 2000; Archer et al., 1999). Additionally, placing selection pressure on FCR may result in changes in component traits associated with selecting based upon a ratio (Hoque et al., 2006). A more desirable selection tool to improve animal efficiency is one that has a favorable or negligible impact on other growth and carcass traits.

Residual feed intake has been studied as an additional measure of production efficiency (Koch et al., 1963). Residual feed intake (RFI) is the deviation between actual and predicted intake based upon the regression of feed intake on average daily gain and mid-test metabolic body weight. RFI has been shown to have a favorable relationship with FCR and intake measures (Jensen et al., 1992; Herd and Bishop, 2000; Hoque et al., 2006; Nkrumah et al., 2007). Genetic analysis has shown that genetic residual feed intake is highly correlated with phenotypic residual feed intake (r=0.95), indicating that they may be thought of as the same trait (Hoque et al., 2006). Additionally, RFI has shown moderate heritability (h^2=0.24), indicating that RFI may contain genetic components that would respond to selection pressure (Hoque et al., 2006).

To capitalize on improving herd efficiency through the genetic variation associated with feed efficiency traits, it is necessary to characterize potential phenotypic differences among beef animals of varying RFI. The objectives of the present study were to determine phenotypic relationships of residual feed intake with growth and carcass performance traits of spring-born Angus bulls.

Materials and Methods

Spring-born Angus bulls were used in this study (n=136). Data were collected over a two-year period at a central bull test station in Yerington, Nevada. In 2007, bulls (n=90) underwent a 62-day test. In 2008, bulls (n=46) underwent a 72-day test. During both test years, bulls received a grower and a finsher ration, following a 28-day adjustment period at the start of the test and a 7-day transition period. The grower ration contained 12% CP, 13.96% CF, 3.73% fat, 1.45 Mcal/kg NEm, and 0.86 Mcal

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NEg on a dry matter basis. The finisher ration was comprised of 10.76% CP, 5.53% CF, 4.03% fat, 1.81 Mcal/kg NEm, and 1.15 Mcal/kg NEg on a dry matter basis. Average start and finish weights were collected. DMI data were collected during the test using the GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). A certified ultrasound technician using an Aloka 500 real-time unit equipped with a 3.5-MHz transducer collected all ultrasound data.

Three different measures of feed efficiency were calculated for each animal. Feed conversion ratio (FCR) was determined as the ratio of DMI to ADG. Partial efficiency of growth (PEG) was computed as the ratio of ADG to DMI for growth (Koch et al., 1963). RFI as defined by Koch et al. (1963) was calculated for each animal as the difference between actual intake and intake predicted by the stepwise linear regression used to determine the order of inclusion of carcass characteristic and the significance of trial to reach the final regression model of DMI on ADG, MMWT ($BW^{0.75}$), and uFT (Statistix9, 2008). Bulls were classified using RFI into low (<-0.5 SD; n=35; RFI=-1.036 kg/d), marginal (±0.5 SD; n=60; RFI=-0.0014 kg/d), or high (>0.5 SD; n=41; RFI=0.887 kg/d) groups (Basarab et al., 2003).

Data were analyzed by ANOVA fitting RFI group as the independent variable (Statistix9, 2008). All pairwise comparisons were made using Tukey HSD (Statistix9, 2008). Relationship between RFI and phenotypic performance traits and carcass traits were established using Pearson Correlation (Statistix9, 2008).

Results and Discussion

Favorable differences in FCR, PEG and daily intake were detected among RFI groups. Low (11.120 kg) RFI grouped bulls exhibited significantly reduced DMI compared to marginal (12.091 kg) and high (12.920 kg) RFI grouped bulls (Table 1). Significant differences in DMI were also detected among marginal and high RFI grouped animals (P=0.00). Lancaster et al. (2005) reported a 15% reduction in feed intake between low and high RFI bulls, despite no detection of differences in ADG and body weight. Similar trends existed for FCR. Low grouped bulls exhibited significantly lower FCR (5.74 kg; P=0.00) relative to marginal (6.43 kg) and high (6.94 kg) grouped bulls. Favorable group differences were apparent for PEG. Low RFI bulls had higher PEG (0.493; P=0.00) than high RFI bulls (0.320). Marginal RFI bulls also had significantly higher PEG (0.380; P=0.00) than high RFI bulls. No significant group differences were detected in on-test weight, off-test weight, or ADG indicating that selecting for reduced RFI may result in improved feed efficiency with minimal impact on growth (Table 1). Residual feed intake has been shown to be genetically and phenotypically independent of its component traits (Arthur et al., 2001). This finding indicates that changes to component traits will not likely result from selection based upon improved RFI.

Analyses of group means for phenotypic carcass characteristics are included in Table 2. No significant group differences were detected in uREA, uFT, or uPIMF. Comparisons made were consistent with the findings of Cardin et al. (2008), indicating low RFI grouped bulls had numerically lower uFT. Robinson and Oddy (2004) suggested that selection for reduced RFI would likely result in decreased subcutaneous fat. Additionally, bulls did not differ based upon RFI group in ultrasound carcass trait EPDs (Table 3). Lancaster et al. (2008) noted that RFI has been weakly correlated with twelfth rib fat thickness. As expected, accounting for body composition in the regression model removed these differences. Basarab et al. (2003) reported that including body composition in the model to determine RFI accounted for more variation in DMI. Lancaster et al. (2009) reported that adjusting RFI for carcass composition only minimally impacts animal ranking in growing animals when compared to using only MMWT and ADG, yet in finishing animals, the relationship between body composition and RFI is stronger suggesting it may be favorable to include body composition in the regression model to calculate RFI.

Phenotypic correlation indicated that RFI was significantly and favorably correlated with FCR (0.490; P=0.00), a finding corroborated by numerous investigations (Jensen et al., 1992; Herd and Bishop, 2000; Hoque et al., 2005; Hoque et al., 2006; Cardin et al., 2008). RFI was also significantly correlated with PEG (-0.898; P=0.00), indicating that selection for reduced RFI would likely result in increased PEG. Lancaster et al. (2005) reported similar findings when RFI was correlated with PEG (-0.85), indicating bulls with low RFI had higher PEG than both marginal and high RFI bulls. RFI was significantly correlated with dry matter intake (0.702; P=0.00). Hoque et al. (2006) and Arthur et al. (2001) reported correlation values of 0.72 and 0.69, respectively, for RFI and DMI. Furthermore, Carstens et al. (2002) and Nkrumah et al. (2004) reported that RFI was significantly correlated with ultrasound back fat (0.22 and 0.19 respectively; P<0.05). Similarly, ultrasound rump fat (0.18; P<0.05) was correlated with RFI as noted by Carstens et al. (2002). No significant correlations were detected between RFI and uREA, or uPIMF. RFI was not significantly correlated with uREA, uPIMF, or uFT EPDs. Similar results were reported by Cardin et al. (2008). Phenotypic correlations of RFI with on-test weight (-0.012; P=0.891) and off-test weight (-0.044; P=0.613) were negligible. These results were corroborated by Jensen et al. (1992), Herd et al. (1999), Hoque et al. (2006) and Lancaster et al. (2008). Results suggest the phenotypic selection for improved RFI appears to result in more efficient animals that consume less but gain the same as their less efficient counterparts without significantly increasing body weight nor affecting carcass composition.

Implications

RFI may serve as an appropriate selection tool for improving feed efficiency without adversely affecting phenotypic growth performance and carcass traits. Research suggests that selection for reduced RFI may result in improved animal efficiency without increasing cow size. Given economic pressures, selection tools with relevance to

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efficiency have significant implications for the commercial cattleman and the modern beef production system. Continued research should be conducted on the economic impact of selecting for reduced RFI.

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Table 1. Mean (SE) phenotypic performance for bulls classified as low (<-0.5 SD; n=35; RFI=-1.036), marginal (±0.5 SD; n=60; RFI=-0.0014 kg/d)), or high (>0.5 SD; n=41; RFI=0.887 kg/d) based upon individual residual feed intake (RFI) values.

	RFI Group		
Trait	Low	Marginal	High
On-Test Wt. (kg)	430.83 (6.70)	435.73 (5.45)	429.54 (5.21)
Off-Test Wt. (kg)	558.25 (7.15)	559.87 (5.62)	549.16 (7.60)
Average Daily Gain (kg/d)	1.97 (0.049)	1.92 (0.040)	1.89 (0.049)
Feed Conversion Ratio (kg feed/kg gain)	5.74 (0.122)[a]	6.43 (0.103)[b]	6.94 (0.133)[c]
Partial Efficiency of Growth (ADG/DMI for growth)	0.492 (0.015)[a]	0.380 (0.004)[b]	0.320 (0.004)[c]
DMI (kg/d)	11.120 (0.165)[a]	12.091 (0.117)[b]	12.920 (0.170)[c]
RFI (kg)	-1.036 (0.098)[a]	-0.0014 (0.033)[b]	0.887 (0.072)[c]

[a,b,c]RFI groups within row without common superscripts differ ($P < 0.05$).

[1]Funded in part by the California State University Agricultural Research Initiative and Snyder Livestock Company, Inc.

Table 2. Mean (SE) carcass performance for bulls classified as low (<-0.5 SD; n=35; RFI=-1.036 kg/d), marginal (±0.5 SD; n=60; RFI=-0.0014 kg/d), or high (>0.5 SD; n=41; RFI=0.887 kg/d) based upon individual residual feed intake (RFI) values.

	RFI Group			
Characteristic	Low	Marginal	High	P-value
Ultrasound Ribeye Area	14.092 (0.193)	14.247 (0.144)	13.766 (0.183)	0.116
Ultrasound Back Fat	0.366 (0.0151)	0.354 (0.0085)	0.373 (0.0133)	0.483
Ultrasound Percent Intramuscular Fat	4.892 (0.183)	4.835 (0.133)	4.963 (0.137)	0.818

Table 3. Mean (SE) carcass character EPDs for bulls classified as low (<-0.5 SD; n=35; RFI=-1.036 kg/d marginal (±0.5 SD; n=60; RFI=-0.0014 kg/d), or high (>0.5 SD; n=41; RFI=0.887 kg/d) based upon individual residual feed intake (RFI) values.

	RFI Group			
EPD	Low	Marginal	High	P-value
Ultrasound Ribeye Area	0.243 (0.029)	0.267 (0.024)	0.211 (0.029)	0.320
Ultrasound Back Fat	0.006 (0.002)	0.005 (0.002)	0.007 (0.002)	0.614
Ultrasound Percent Intramuscular Fat	0.177 (0.036)	0.145 (0.023)	0.149 (0.028)	0.705

Table 4. Pearson correlations of residual feed intake (RFI) with performance traits in bulls.

Trait	RFI (kg/d)	P-value
Initial Weight (kg)	-0.012	0.891
End Weight (kg)	-0.044	0.613
Average Daily Gain	0.000	1.000
Midtest Metabolic Weight (kg)	0.000	1.000
DMI (kg/d)	0.702	0.000
Feed Conversion Ratio (feed/gain)	0.490	0.000
Partial Efficiency of Growth (PEG; ADG/DMI for growth)	-0.898	0.000
Ultrasound Ribeye Area (REA)	-0.125	0.146
Ultrasound Back Fat	0.000	1.000
Ultrasound Percent Intramuscular Fat (PIMF)	0.037	0.670
REA EPD	-0.090	0.299
PIMF EPD	-0.053	0.538
Back Fat EPD	-0.002	0.979

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RELATIONSHIPS BETWEEN SIRE CALVING EASE EPD AND PROGENY CARCASS PERFORMANCE

C. T. Pendley, C. M. McAllister, S. E. Speidel, D.H. Crews, Jr., J. D. Tatum and R. M. Enns

Department of Animal Sciences, Colorado State University, Fort Collins 80523

ABSTRACT: Crossbreeding systems commonly make use of terminal sires to improve progeny performance with respect to carcass merit. Direct calving ease (CE) is rarely considered in these situations as terminal sires are typically mated to mature cows. There is also potential to generate heterosis by using terminal sires for mating to first-calf heifers. Based on limited study, CE may be unfavorably related to carcass yield. Therefore, the objective of this study was to estimate the effect of sire CE EPD on USDA yield grade (YG), its components and marbling score in crossbred steer and heifer progeny of terminal yield. Carcass data from steers (n=205) and heifers (n=193), sired by four registered Charolais bulls across four years were obtained from Colorado State University's Eastern Colorado Research Center. The breed composition of dams was primarily Angus and Angus cross. Steers and heifers were assigned to either high (n = 210) or low (n = 188) CE group on the basis of CE EPD of their sire. Average CE EPD for sires was 2.2 and -0.8 in the high and low groups, respectively. Steers and heifers were harvested at an average age of 417 d and carcass data including hot carcass weight (HCW), 12th rib subcutaneous fat depth (FAT), longissimus muscle area (REA) and marbling score (MARB) were recorded following routine harvest procedures. Records for YG were computed from component carcass data. Data were analyzed using a general linear model that included the fixed effects of contemporary group (defined as the combination of sex and harvest date), sire CE group, and a linear covariate of harvest age.. Differences in group least squares means were 1.8 cm^2 for REA ($P<0.06$) and 6.3 kg for HCW ($P<0.03$). The groups did not significantly differ ($P>0.58$) in carcass FAT, MARB, or YG. These results suggest that selection for increasing direct genetic merit for CE may result in larger REA and heavier HCW in Charolais-sired crossbred steers and heifers, but would not affect USDA yield grade or marbling score, although this sire sample is limited and further study is required.

Key words: beef cattle, calving ease, carcass, Charolais, terminal sire

Introduction

The beef industry has long recognized the importance of calving ease due to its impact on calf survivability and subsequent cow post partum uterine involution. Calving ease (**CE**) is a large economic component of cow herd profitability and thus should be an important selection criterion considered when making breeding decisions (Golden et al. 2000).

Often, selection pressure is not placed on calving ease in terminal crossbreeding systems because females are not retained for breeding purposes and the CE EPD primarily accounts for calving difficulty in first calf heifers. In the Charolais breed CE is calculated as an average difference of unassisted births of the sire's calves when bred to first-calf heifers. (AICA, 2008)

There is an unfavorable relationship between CE and post-natal growth; the larger the calf, the faster and more efficient they grow. This has been documented by Bennett et al. (2008). With the positive correlation between CE and growth, results have not been published associating CE with carcass merit.

Potential to generate heterosis through the use of terminal sires on first-calf heifers exists, but is often overlooked due to the added cost associated with calving difficulty and heifer fall-out due to dystocia. (Bennett et al., 2008) Little research has focused on the effect terminal sires with high CE EPD have on carcass merit of their progeny. Therefore, the objective of this study was to estimate the effect of sire CE EPD on USDA yield grade (YG), its components, and marbling score in crossbred steer and heifer progeny of Charolais sires.

Materials and Methods

These data were collected under Colorado State University's Animal Care and Use Committee guidelines. Records from 398 animals, both steers (n=205) and heifers (n=193), with carcass observations, were obtained from Colorado State University's Eastern Colorado Research Center in Akron, Colorado. The carcass measurements included hot carcass weight (**HCW**), 12th rib subcutaneous fat depth (**FAT**), longissimus muscle area (**REA**) and

marbling score (**MARB**) and were recorded using routine post-harvest procedures. Steers and heifers were harvested at an average age of 417 d (SD = 16.3). Animals were sired by four registered Charolais bulls; the progeny were placed into either high (n=210) or low (n=188) calving ease groups based on their sires' CE EPD. The sires were selected primarily on their carcass EPD with no selection criteria for CE EPD. The dams were crossbred commercial cows of primarily British influence.

Carcass data was analyzed using a general linear model (SAS Ver. 9.2, Cary, NC). The model included the fixed effects of contemporary group (defined as the combination of sex and harvest date), sire CE group, and a linear covariate of harvest age. The formation of contemporary groups in this manner resulted in 24 unique groups. Least squares mean estimates were calculated for each of the carcass traits using the model equation:

$$y_{ijkl} = b_j CG_j + b_k G_k + b_l HA_l + e_1$$

where, y was the carcass trait measured on animals, CG was the contemporary group, G was the sire group based on sires' CE EPD, HA was the harvest age of the individual, and e was a vector of random residual errors specific to each observation in y.

Results and Discussion

Summary statistics for carcass observation are summarized in Table 1 for HCW, REA, FAT, MARB, and YG. The USDA YG grade was calculated using HCW, REA, and FAT (AMS 2001) .

Table 1: Summary statistics for progeny carcass traits[1]

	N	Mean	SD	Min[2]	Max[2]
HCW	312	372.9	32.9	257.0	454.5
REA	398	92.5	8.3	65.2	120.3
FAT	398	1.34	0.41	0.41	3.05
MARB	398	3.95	0.70	1.80	7.00
YG	398	2.99	0.57	1.07	4.70

[1] HCW = Hot Carcass Weight (kg); REA=Longissimus Muscle Area (cm^2); FAT= Subcutaneous Fat depth (cm); MARB=Marbling Score; YG= USDA Yield Grade
[2] SD= Standard Deviation; Min= Minimum Value; Max= Maximum Value

Least squares means for high and low sire CE EPD groups are shown below in Table 2.

Table 2: Least squares means for progeny carcass traits[1]

	N	LSM±SE[2]	P-value[4]
HCW			
High[3]	139	371.62±1.84[a]	< 0.03
Low[3]	173	365.29±2.07[b]	
REA			
High	210	92.89±0.54[a]	< 0.06
Low	188	91.11±0.71[b]	
Fat			
High	210	1.28±0.027[a]	< 0.91
Low	188	1.28±0.035[a]	
MARB			
High	210	3.93±0.047[a]	< 0.53
Low	188	3.89±0.061[a]	
YG			
High	210	2.97±0.037[a]	< 0.69
Low	188	2.99±0.048[a]	

[1] HCW = Hot Carcass Weight (kg); REA=Longissimus Muscle Area (cm^2); FAT= Subcutaneous Fat depth (cm); MARB=Marbling Score; YG= USDA Yield Grade
[2] LSM= Least Squares Means
[3]High= Calves sired by high calving ease sires; Low=calves sired by low calving ease sires
[4]P-value is representative of LSM group differences
[a,b]=group means without common superscript letter differ, P< 0.05

Results of this analysis were unanticipated. Typically, calving ease is closely correlated with birth weight as shown by both Meijering (1984) and Koots et al. (1994). Birth weight has also been shown to have a high correlation with carcass weight (Eriksson et al. 2004) Following these studies, one can hypothesize that low calving ease sires would produce calves with heavier HCW and larger REA. The results of this study contradict this hypothesis. Average CE EPD for sires was 2.2 and -0.8 for the high and low groups, respectively. Differences between the calving ease groups (High - Low) were 1.8 cm^2 for REA (P<0.06) and 6.3 kg for HCW (P<0.03), suggesting that calves sired by high calving ease sires had larger REA and heavier HCW than those sired by low calving ease sires. The other carcass traits measured, FAT, MARB, and YG were -0.005 (P=0.91), 0.049 (P=0.53), -0.025 (P=0.69), respectively, for the between group differences, they did not significantly differ from each other. There appears to be no negative effects to including CE as a sire selection criterion. In fact, there is a positive relationship with regard to REA and HCW of progeny with high CE EPD sire. This is a small sample size with limited sires. This study serves as a

pilot study that warrants more investigation for capitalizing on heterosis in first calf heifers.

Implications

These results suggest sires with a high calving ease EPD produce progeny with heavier hot carcass weights and larger rib-eye areas. USDA yield grade, 12th rib subcutaneous fat depth, and marbling score did not differ with regard to sire calving ease EPD. Further research is warranted to investigate these effects.

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RELATIONSHIPS OF EXIT VELOCITY AND AVERAGE CHUTE SCORE WITH CARCASS TRAITS IN FEEDLOT STEERS

A.R. Pepper[1], R.M. Enns[1], R.L. Weaber[2], H. Van Campen[3], G.H. Loneragan[4], J.L. Salak-Johnson[5], C.C.L. Chase[6], R.K. Peel[1], J.J. Wagner[1], D.H. Crews, Jr.[1], and E.J. Pollak[7]

[1]Department of Animal Sciences, Colorado State University, Fort Collins; [2]Division of Animal Science, University of Missouri, Columbia; [3]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins; [4]Department of Agricultural Sciences, West Texas A&M University, Canyon; [5]Department of Animal Sciences, University of Illinois, Urbana; [6]Department of Biology and Microbiology, South Dakota State University, Brookings; [7]Department of Animal Sciences, Cornell University, Ithaca

ABSRACT: The objective of this study was to estimate relationships between temperament, as measured by exit velocity and chute score, and carcass trait performance. Crossbred steers (n=1,551) from three operational units of a single ranch were transported approximately 8 hours from western Nebraska to a commercial feedlot in southeast Colorado over three days. Steers were housed overnight in receiving pens before being processed and allocated to their final pens (n=6) for the feeding period. Exit velocity and chute score were measured at receiving (**EV**; m/s, **CS**) and approximately 75 d later at re-implant (**EV_RI**; m/s, **CS_RI**). Animals were harvested and carcass data collected after 225 d on feed. Analyses were conducted using a mixed effects model via the MIXED procedure of SAS. Analyses evaluated the associations of EV, EV_RI, CS, and CS_RI independently on hot carcass weight (**HCW**), fat thickness (**FAT**), ribeye area (**REA**), kidney, pelvic and heart fat (**KPH**), marbling score (**MS**), and USDA yield grade (**YG**). All models included the fixed effect of ranch-pen class, and a random animal effect. An increase of 1m/s in EV was associated with a 2.69 kg decrease ($P < 0.05$) in HCW. An increase of 1 m/s in EV_RI revealed an associated decrease in HCW of 2.29 kg ($P < 0.01$). Similarly a one unit increase in CS was associated with a decrease in marbling score (-4.3; $P < 0.05$), and a 0.03% increase ($P < 0.10$) in KPH. All other regression estimates were not significantly different than zero ($P > 0.1$). These results suggest that cattle with more excitable temperaments have lighter HCW, lower meat quality, and higher KPH percentage.

Key Words: beef cattle, carcass traits, chute score, exit velocity

Introduction

Temperament has been defined as an animal's behavioral response to handling (Nkrumah et al., 2007). Many measures of temperament are very subjective and Burrow et al. (1988) developed flight speed as an objective measurement of temperament. Flight speed has been defined as the time it takes for an animal to travel a predetermined distance after exiting a confined area (Burrow et al., 1988).

Docile animals are appreciated by producers in terms of ease of handling; however this is not the only reason selection may be practiced for more favorable temperaments. Tulloh (1961) reported a favorable relationship between live weight and docility in cattle. Tulloh (1961) stated that selection based on live weight would likely lead to an improvement in temperament. Burrow (1997) stated that temperament is moderately heritable, so selection for more docile animals should show progress. Burrow and Dillon (1997) suggested that docile animals grow faster in a feedlot than more excitable animals because of increased feed intake, or because more excitable animals might partition nutrients differently due to avoidance behavior.

While research suggests that temperament measures are heritable and related to growth measures, little information exists relative to the relationship of temperament and carcass performance; therefore, the objective of this study was to determine the relationship between temperament, as measured by exit velocity and chute score, and carcass trait performance.

Materials and Methods

Crossbred steers (n=1,551), from three operational units of a single Nebraska ranch were transported approximately 8 hours from western Nebraska to the Southeastern Colorado Research Center (**SECRC**) in Lamar, CO. Steers were processed over three days, and maintained for the duration of the study at a commercial feedlot immediately adjacent to the SECRC. Animals were processed at receiving and approximately 75 days later, at those times the following observations were recorded: exit velocity (**EV** - exit velocity at receiving**; EV_RI** - exit velocity at re-implant), chute score (**CS** - chute score at receiving**; CS_RI** - chute score at re-implant). After 225 days in the feedlot, animals were harvested and hot carcass weight (**HCW**, kg), fat thickness (**FAT**, cm), ribeye area (**REA**, cm^2), kidney, pelvic and heart fat (**KPH**, %), marbling score (**MS**), and USDA yield grade (**YG**) information collected as per normal carcass data collection procedures. Flight speed was measured using two infrared electronic triggers to start and stop an electronic time recording device, the first as the

steer left the squeeze chute and a second 1.83 m away from the first. Exit velocity was calculated based on those timings. Chute scores (1-6; 1 = docile, 2 = restless, 3 = nervous, 4 = flighty, 5 = aggressive, 6 = very aggressive) were obtained by two trained personnel, during processing, in accordance with Beef Improvement Federation guidelines (BIF, 2002). Table 1 lists summary statistics for carcass and temperament traits.

Initial analyses included the calculation of Pearson correlation coefficients between temperament and harvest observations via SAS (SAS Institute., Inc., Cary, NC). Additional analyses, involved fitting harvest observations as dependent variables in a mixed model analysis (SAS Institute., Inc., Cary, NC) that included the fixed effects of unit-pen and a random animal effect. Additionally, each temperament variable was independently added to the model as a covariate to determine the magnitude of the effect of the temperament trait on the harvest outcome. This resulted in a regression coefficient estimate for each model that regressed the harvest trait observation on the temperament trait after adjusting for the other fixed effects in the model.

Data sifting procedures were used to eliminate EV and EV_RI less than zero or greater than ten m/s as these outliers, greater than 4 standard deviations from the mean, were assumed to be attributable to measurement error. Hot carcass weights greater than 5 standard deviations from the mean were also omitted from the final data set analyzed and were assumed to be attributable to measurement error.

Results and Discussion

Pearson correlation coefficients are presented in Table 2 for HCW and YG. Exit velocity at receiving was significantly correlated with HCW ($r = -0.06$, $P < 0.05$) although the magnitude of the correlation was small. Exit velocity at re-implant was significantly correlated with HCW ($r = -0.10$, $P < 0.001$) and also with YG ($r = -0.05$, $P < 0.1$). Chute score at receiving was significantly correlated with HCW ($r = 0.05$, $P < 0.1$). All other correlation estimates were not significantly different from zero ($P > 0.1$). These are of the same direction as correlations reported by Kadel et al. (2006) who found genetic correlations between temperament measures and meat quality to be moderate (approximately 0.28 and 0.2; CS and flight speed respectively), but are much higher than those reported here.

The magnitude of the associations of temperament measures with harvest measures suggest that improved temperament would have favorable effects on several carcass traits. An increase in EV of 1 m/s was associated with a decrease in HCW of 2.69 kg ($P < 0.05$) and the later measurement of EV_RI indicated that an increase of 1 m/s in EV_RI was associated with a decrease in HCW of 2.29 kg ($P < 0.01$). Increases in CS of one unit were associated with a 4.3 unit decrease ($P < 0.05$) in MS, and a 0.03% increase ($P < 0.10$) in KPH, respectively. All other models were not significantly different from zero ($P > 0.10$). King et al. (2006) found temperament traits to have no affect on YG, MS, KPH, and FAT. This is in agreement with what was found in this study for YG and FAT, but is in disagreement with the results of this study for MS, and KPH.

Burrow et al. (1988) reported heritability estimates for flight speed from 0.26 to 0.54, whereas Kadel et al. (2006) reported a heritability estimate of 0.31. Flight speed has been assumed to be a measure of the intrinsic fear of animals that could be considered an appropriate and reliable measurement of temperament (Petherick et al., 2002). While these measures of temperament have been reported to be heritable, flight speed and chute scores cannot be considered genetically equivalent traits (Kadel et al., 2006) and hence both were measured in this study.

In feedlots it is inevitable that cattle are going to be handled frequently due to processing, health status inspection, data collection, and feeding. More docile animals have the ability to gain faster than flighty animals . While greater live weight gain may be very high on most feedlot priority lists, it's worth it to note that tenderness is high on the priority list of consumers. The temperament of cattle has been found to have a significant effect on carcass tenderness (Voisinet et al., 1997).

The effect of docility on live weight is agreed upon as being favorable (Behrends et al., 2009; Burrow et al., 1997; Tulloh, 1961). However, when it comes to carcass traits results are conflicting. Many have found there to be no effect of temperament on carcass traits (Burrow and Dillon, 1997; Kadel et al., 2006; King et al., 2006; Petherick et al., 2002) in contrast to the results reported herein. Behrends et al. (2009) found exit velocity to be significantly related to REA and YG. Voisinet et al. (1997) also found a significant relationship between temperament and yield grades.

Implications

These results suggest that there is a correlation between temperament scores and some carcass traits in cattle. Cattle with more excitable temperaments have lighter HCW, lower marbling, and higher KPH percentage. While the results of the association of temperament with carcass traits are conflicting, none of the studies shown herein have found a negative effect of docile animals on carcass traits. These results suggest that producers should select for more docile animals, if they are able to do so.

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Table 1. Summary of Statistics for Temperament and Carcass Traits.[1]

Trait	N	Mean	SD
CS[2]	1551	2.97	0.86
CS_RI[2]	1513	2.50	0.90
EV[2], m/s	1488	2.94	0.77
EV_RI[2], m/s	1465	3.15	1.00
Fat thickness, cm	1259	0.51	0.16
HCW, kg	1288	358.49	32.42
KPH, %	1288	3.02	0.50
Marbling	1258	40.66	5.34
Ribeye area, cm^2	1269	12.97	1.41
Yield grade	1259	2.51	0.72

[1]Temperament traits = CS, CS_RI, EV, and EV_RI; carcass traits = fat thickness, Hot Carcass Weight (HCW), kidney, pelvic and heart fat (KPH), marbling, ribeye area, and USDA yield grade.

[2]CS = chute score at receiving; CS_RI = chute score at re-implant; EV = exit velocity at receiving; EV_RI = exit velocity at re-implant.

Table 2. Pearson Correlation Coefficients of Exit Velocity and Chute Score with Hot Carcass Weight (HCW) and USDA Yield Grade (YG).

Trait	CS[1]	EV[1]	EV_RI[1]
HCW, kg	0.05	-0.06	-0.10
YG	-0.01[2]	-0.02[2]	-0.05

[1]CS = chute score at receiving; EV = exit velocity at receiving; EV_RI = exit velocity at re-implant.

[2]$P > 0.1$

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RELATIONSHIP BETWEEN ULTRASONICALLY-MEASURED BEEF COW CARCASS TRAITS AND LIFETIME PRODUCTIVITY

L. A. Pacheco*, J. R. Jaeger†, J. Minick-Bormann*, and K. C. Olson*

*Kansas State University, Manhattan, KS 66506
†Western Kansas Agricultural Research Centers, Kansas State University, Hays, KS 67601

ABSTRACT: Intramuscular fat (IMF) and longissimus muscle depth (LMD) are commonly used in the beef industry to aid in replacement heifer selection. The objective of our study was to determine if ultrasonically-measured IMF and LMD were related to lifetime cow productivity and progeny performance. Angus x heifers (n = 160) were managed as a contemporary group and developed in a drylot until breeding at 14 mo of age. Heifer IMF and LMD were measured at approximately 1 yr of age. Each year, females were mass-mated following estrous synchronization and exposed to bulls 10 d later for the remainder of a 45 d breeding season. Heifers were managed in a spring-calving, native range-based production system with a 12 mo calving interval for the duration of the 4 yr study (2004-2007). Animals were examined for pregnancy yearly in August and non-pregnant females were removed from the herd. Cow IMF and LMD were categorized into high, medium, and low groups. Cow IMF, LMD, IMF group (IMFG), and LMD group (LMDG) were analyzed using a fixed general linear statistical model. Pregnancy rate was not related to cow IMF, LMD, or IMFG ($P > 0.05$); however, more cows in the high and medium LMDG were pregnant than cows in the low LMDG ($P < 0.04$). Calving interval was not related to cow IMF, LMD, IMFG, or LMDG ($P > 0.05$). Calf BW at birth was not related to dam IMF, LMD, IMFG, or LMDG ($P > 0.05$). Calf BW at weaning was not related to dam LMD, IMFG, or LMDG ($P > 0.05$); however, calf BW at weaning increased as dam IMF increased ($P < 0.05$). These data were interpreted to suggest that greater cow IMF was associated with greater progeny BW at weaning. In contrast, cow IMF was not related to pregnancy rate, calf BW at birth, or calving interval. Moreover, cow LMD and IMFG were not related to pregnancy rate, calf birth weights, calf weaning weights, or calving interval. Further analysis of IMF and LMD and their effect on cow productivity and progeny performance appears warranted as more production records are obtained from these females.

Key W ords: Beef Cows, Intramuscular Fat, Longissimus Muscle.

Introduction

Ultrasound is widely used in seedstock production, commercial operations, and in feedyards to predict carcass merit. It has also been used to assess the value of individuals as parents in the seedstock industry. Ultrasound has many advantages as a technique to evaluate body composition: it is relatively inexpensive; it produces results more rapidly compared to progeny testing programs; and data are less prone to selection bias than direct carcass data collection. Studies have demonstrated that ultrasound measures of ribeye area (**REA**), (Perkins et al., 1992; Herring et al., 1994) and proportion of intramuscular fat (**IMF**), (Reverter et al., 2000; Hassen et al., 2001) are accurate predictors of their corresponding carcass traits in fed slaughter cattle. Thus, average heritability estimates of ultrasonically-measured REA and IMF are moderate to high. Moderate-to-high heritability allows seedstock breeders to select replacement animals with confidence based on ultrasound measurements.

A large body of research has evaluated the use of sire and ultrasound measures as a predictor of progeny carcass measurements and growth. In contrast, little research has examined the use of ultrasonically-measured compositional traits as a means to predict cow productivity and subsequent progeny performance. Available data in this area appear to be limited to actual carcass evaluation; Davis and coworkers (1983) concluded that dam carcass traits at 240 d of age were not associated with progeny efficiency and suggested that identification of heifers that will produce offspring with superior efficiency cows is difficult.

The objective of our experiment was to examine the use of ultrasound measures of IMF and longissimus muscle depth (**LMD**) as a means to predict lifetime cow productivity and progeny performance. Specifically, we wished to determine whether ultrasound measurements of IMF and LMD obtained from yearling heifers were related to calf birth weight, calf weaning weight, cow pregnancy rate, and calving interval.

Materials and Methods

Animals and Data Collection. Angus-cross heifers (n = 160) were retained from the KSU Agricultural Research Center-Hays herd or purchased from two sources with similar genetics and breeding seasons and managed as a contemporary group. All procedures used in the care, handling, and sampling of animals in our study were approved by the Kansas State University Institutional Animal Care and Use Committee. Females were developed in a drylot and had *ad libitum* access to a grower diet and clean water. At approximately 1 yr of age, measurements of heifer IMF and LMD at the 12^{th} to 13^{th}-rib interface were obtained by an experienced technician. Ultrasound images were generated using an Aloka 500V (Aloka Co., Ltd,

Wallingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-125 mm window). Images were collected by a single technician with software from the Cattle Performance Enhancement Company (**CPEC**, Oakley, KS). Backfat thickness, LMD, and IMF were estimated with procedures that incorporated image analysis software (Brethour, 1994) integral to the CPEC product. Marbling scores were coded such that 4.0 = slight^{00} (low select) and 5.0 = small^{00} (low choice).

Following breeding at approximately 14 mo of age, heifers were managed in a spring-calving, native range-based production system with a 12 mo calving interval for the duration of the 4 yr study (2004-2007). Each year, females were mass-mated following estrous synchronization and exposed to Angus bulls 10 d later for the duration of a 45-d breeding season. Pregnancy rate to AI was determined 31 to 35 d after fixed-time AI with transrectal ultrasonography. Cows were examined for pregnancy in August each year via rectal palpation and non-pregnant females were removed from the herd. Calves were weighed at birth and weaning. Weaning weights were adjusted for age of calf, age of dam, and sex of calf.

Statistics. Measurements of IMF and LMD from yearling heifers were categorized into low, medium, and high groups (< 3.88%, 3.88 to 5.33%, and > 5.33%, respectively, for IMF and < 43.80 cm, 43.80 to 52.02 cm, and > 52.02 cm, respectively, for LMD). All data were analyzed using the Mixed and Logistic procedures of SAS (SAS Inst. Inc., Cary, NC). The Mixed model included fixed effects for year, calf sex, IMF group (IMFG), and LMD group (LMDG) on calf birth wt, calf weaning wt, and calving interval. The Logistic model included fixed effects for year, calf sex, IMFG, and LMDG on pregnancy rate. Data were presented as least squares means with differences being considered significant at $P \leq 0.05$.

Results and Discussion

Pregnancy rate was not related to cow IMF, LMD, or IMFG ($P > 0.05$); however, more cows became pregnant in the high and medium LMDG compared to the low LMDG ($P < 0.04$; Table 1). Heavier muscling may be associated with greater fertility. In contrast, heavier muscling may have been secondary to a superior plane of nutrition between weaning and breeding. Calving interval was not related to cow IMF, LMD, IMFG, or LMDG ($P > 0.05$).

Calf BW at birth was not related to dam IMF, LMD, IMFG, or LMDG ($P > 0.05$). Calf 205-d adjusted weaning BW was not related to dam LMD, IMFG, or LMDG ($P > 0.05$); however, calf 205-d adjusted weaning BW increased as dam IMF increased ($P < 0.05$). These data were interpreted to suggest that heifer IMF was associated with greater progeny BW at weaning. Based on these data, each 1% increase in IMF was associated with a 3.9 kg increase in calf BW at weaning.

Greater heifer IMF at weaning may be related to greater growth efficiency by progeny. Change in cow weight:height from calving to weaning was positively related to calf growth efficiency (Davis et al., 1983; Davis et al., 1985). Conversely, several studies have reported negative relationships between dam weight gain or weight:height and pre-weaning ADG and F:G of progeny (Gregory et al., 1950; Brinks et al., 1962; Todd et al., 1968; Kress et al., 1969; Carpenter et al., 1972; Hohenboken et al., 1973).

Cow IMF was not related to pregnancy rate, calf BW at birth, or calving interval. Moreover, cow LMD and IMFG were not related to pregnancy rate, calf BW at birth, calf 205-d adjusted weaning BW, or calving interval.

Calf 205-d adjusted weaning BW increased numerically as LMDG and IMFG increased. Hohenboken and colleagues (1973) reported that there were small positive correlations between cow size at parturition and calf size at birth and weaning. Cow size at parturition would be due, in part, to heaviness of muscling; therefore, LMD should be related to BW at weaning. Arnold and coworkers (1991) concluded that when ultrasonically-measured REA was adjusted to a constant age, there was a positive genetic correlation to 205-d adjusted weaning weight. Use of ultrasound for REA measurement would provide a potential rate of change of 0.32 cm^2/yr, which is nearly 2-fold greater than when testing 10 progeny/sire (0.17 cm^2/yr).

Further analyses of heifer IMF and LMD and their effects on lifetime cow productivity and progeny performance appear to be warranted as more production records are obtained from these females.

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Table 1. Relationship between longissimus muscle depth (LMD) in heifers at a year of age and production measures collected from 2 to 5 years of age.

	Longissimus muscle depth group*		
Trait (mean ± S.E.)	Low (< 43.80 cm)	Medium (43.80–52.02 cm)	High (> 52.02 cm)
Calf BW at birth, kg	37.2 ± 1.0	35.9 ± 0.3	35.6 ± 0.7
Calf 205-d adjusted weaning BW, kg	232.8 ± 6.6	239.0 ± 4.4	243.0 ± 5.7
Calving interval, d	350.6 ± 5.1	343.6 ± 4.0	345.6 ± 4.9
Pregnancy rate, %	78.0[a]	91.0[b]	88.0[b]

*Longissimus muscle depth (LMD) was measured at approximately a year of age with ultrasound and heifers were categorized into high, medium or low LMD groups.

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

Table 2. Relationship betweem amount of intramuscular fat in heifers at a year of age and production measures collected from 2 to 5 years of age.

	Intramuscular fat group*		
Trait (mean ± S.E.)	Low (< 3.88%)	Medium (3.88–5.33%)	High (> 5.33%)
Calf BW at birth, kg	36.0 ± 0.7	37.0 ± 0.3	36.1 ± 0.7
Calf 205-d adjusted weaning BW, kg	235.9 ± 5.3	239.3 ± 4.5	244.1 ± 5.8
Calving interval, d	344.0 ± 4.7	345.8 ± 4.2	342.4 ± 4.7
Pregnancy rate, %	92.7	89.4	84.9

*Intramuscular fat (IMF) was measured at approximately a year of age with ultrasound and heifers were categorized into high, medium or low IMF groups.

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GENETIC PARAMETERS FOR COW WEIGHT AND HEIGHT USING A REPEATABILITY MODEL IN AMERICAN ANGUS CATTLE

M.G. Dib[1], L. D. Van Vleck[1,2] and M. L. Spangler[1]

[1]University of Nebraska, Lincoln, NE, [2]ARS-USDA Lincoln, NE

ABSTRACT: Estimates of genetic parameters were obtained from two samples of weights and heights of mature cows provided by the American Angus Association. The first sample consisted of 23,658 records for mature weight (MWT) and 13,012 for mature height (MHT) and the second sample consisted of 23,698 records for MWT and 13,310 for MHT. The four-generation pedigree file included 43,105 animals for the first sample and 44,141 animals for the second sample. Range in ages when cows were weighed was 2 to 11 years at the time of measurement. Variance components were estimated using the MTDFREML programs. Univariate and bivariate analyses were used to estimate genetic parameters for MWT, MHT, and the corresponding genetic correlation. The model included fixed effects of cow age and random cow permanent environmental, contemporary group (herd and year) and residual effects. Heritability estimates (SE) within contemporary group were 0.45 (0.012) for MWT and 0.64 (0.018) for MHT for sample 1 and 0.48 (0.011) for MWT and 0.62 (0.018) for MHT for sample 2. Estimates of repeatability were 0.64 and 0.77 for MWT and MHT, respectively for sample 1 and 0.66 and 0.70 for MWT and MHT, respectively for sample 2. The genetic and permanent environmental correlations between MWT and MHT were 0.80 and 0.75, respectively for sample 1 and 0.83 and 0.69 for sample 2. The estimates of genetic parameters will be used to estimate genetic changes in MWT and MHT from the complete data file.

Key Words: Angus, Cow Height, Cow Weight, Heritability

Introduction

Cow weights and cow heights have been used to estimate lifetime growth curves (Johnson et al., 1990), influence of body size on efficiency (Morris and Wilton, 1976), production including maintenance requirements (Morris and Wilton, 1986), cow-calf profitability, reproduction (Olson et al., 1994), and cull cow value. Mature size can potentially impact the profitability of beef enterprises and thus should be considered in selection programs. Previous direct heritability estimates have been generally moderate to high using various models (Northcutt and Wilson, 1993; Kaps et al., 1999; Rumph et al., 2002).

The objective of the current study was to estimate genetic parameters and (co) variance components for mature weight and mature height of Angus cows using a repeatability model as a first step to estimate genetic trends.

Materials and Methods

The cow weights and heights data and pedigree files used were supplied by the American Angus Association. Two samples were obtained from the complete data file based on the last digit of the herd code. The first sample contained 23,658 MWT and 13,012 MHT records (Table.1). The second sample contained 23,698 MWT and 13,310 MHT records. The four-generation pedigree files included 43,105 and 44,141 animals for samples 1 and 2, respectively. The records were from cows born between 1983 and 2006. The range in ages when cows were weighed was 2 to 11 years with the majority (80%) of records coming from cows between 2 and 6 years of age. Cows on average had 1.7 records for MWT.

Animal Model

In matrix notation, the linear model equation for the vector of observations, y, is:

$$y = Xb + Z + Q + W + e,$$

where y is the vector of observed records, b is a vector of fixed effects of age when measured; a is a vector of random additive genetic effects; c is a vector of random contemporary group effects; W is a vector of random permanent environmental effects of the cows; X, Z, and Q and W are incidence matrices relating Xb, Q, Z, and W to y; and e is a vector of random residual effects. Univariate and bivariate analyses were used to estimate genetic parameters for MWT and MHT, with Henderson's (1977, 1984) augmented mixed model equations and the inverse of the four generation relationship matrix (Henderson, 1976; Quaas, 1976). Estimates were obtained using the MTDFREML programs (BOLDMAN et al., 1995).

Results and Discussion

Estimates of variance and covariance components, heritability and repeatability for samples 1 and 2 are reported in Tables 2 and 3. Estimates of heritability for MWT were similar to those from previous reports. Johnson

et al. (1990) estimated heritability to be 0.38 but it was associated with a large standard error (0.30). Kaps et al. (1999) reported an estimate of 0.59 using a two-trait animal model with adjusted weaning weight and repeated mature weights, with fixed effects of weaning and cow contemporary groups, and direct genetic, maternal genetic and maternal permanent environment as random effects. Rumph et al. (2002) obtained heritability estimates ranging between 0.53 and 0.69 using 6 different models with the most optimal model being the full model that included direct and maternal genetic, direct permanent environment and maternal permanent environment as random effects.

For mature weight, Northcutt and Wilson (1993) reported estimates of heritability of 0.45 (0.10) and 0.48 (0.10) for weights adjusted for body condition score and unadjusted for body condition score, respectively, using a two-trait model for mature weight and mature height.. For mature height, Northcutt and Wilson (1993) reported estimates of heritability of 0.83 (0.11) using the same model. Estimates of variance components and heritability reported by Northcutt and Wilson (1993) for the two-trait model were similar to those from the single trait analyses in the present study. Estimates of repeatability were 0.64 and 0.65 for cow weight for samples 1 and 2 and were 0.77 and 0.70 for cow height. Contemporary groups accounted for about 50% of phenotypic variance for both MWT and MHT.

Genetic correlations between weight and height were strong and positive (table 4). Previous studies have reported similar results, as shown by Northcutt and Wilson (1993) who estimated the Spearmen rank correlation between weight and height to be 0.94. The permanent environmental correlations were also high, ranging from 0.69 to 0.75. In comparison with previous studies it may be important to note that in the present study, the number of animals with records and in the pedigree file were larger. Some of the small differences in estimates also may be caused by differences in models or statistical methods used in the analyses.

Implications

Results from the current study, as expected, show that both MWT and MHT would respond favorably to selection and that changing one would lead to correlated response in the other. Selection would be more accurate for MHT than for MWT because heritability is greater and because less variation is due to permanent environmental effects. These results also show that selection for the total animal effect (genetic plus permanent environmental values) would be considerably more accurate than selection for breeding value especially for MWT. The similarity of estimates of variance components for the two samples show that the data can be pooled in the second step of this project using the complete data file to determine if selection has been to increase or decrease MWT and MHT.

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POPULATION STATUS OF MAJOR U.S. SWINE BREEDS

C.S. Welsh*[1], H.D. Blackburn[1], C. Schwab[2]
[1]USDA-ARS-National Animal Germplasm Program, Fort Collins, CO, USA
[2]National Swine Registry (NSR), West Lafayette, IN, USA

ABSTRACT: Globally, genetic diversity of livestock populations is contracting. Knowing the true extent of the contraction is needed to develop effective conservation strategies. To accomplish this goal, pedigree records were obtained for: Duroc (n = 878,480), Hampshire (n = 744,270), Landrace (n = 126,566), and Yorkshire (n = 727,268) from NSR, and Berkshire (n = 116,758 American Berkshire Association). Number of registrations peaked in 1990 for all breeds except Berkshire and all have been declining in the current decade. Presently, more than 99% of all pigs are inbred with the majority having inbreeding less than 10%. The range for percent of animals that are more than 25% inbred ranged from 1.16% for Yorkshire to 6.09% for Berkshire. The highest inbreeding for all animals within a breed ranged from 51% for Landrace and 65% for Yorkshire. Sires were grouped into ten percentiles based on number of great-grandprogeny (GGP); the top percentile for all breeds accounted for more than 75% of all GGP. Sixty percent of all sires produced less than 1% of all GGP, indicating few males are responsible for the majority of future generations, thus narrowing the genetic base. Generation numbers were computed with the founders defined as having unknown parents, assigned as generation zero. Generations ranged from 17 to 19 per breed with a generation interval ranging from 1.65 yr for Berkshire to 2.21 yr for Yorkshire. Mean inbreeding (%) at generation 17, inbreeding rate of increase per generation, and effective population size were 12.3, 0.0065, and 77 for Berkshire, 11.8, 0.0044, and 113 for Duroc, 6.8, 0.0046, and 109 for Hampshire, 17.9, 0.0067, and 74 for Landrace, and 8.0, 0.0044, and 113 for Yorkshire, respectively. The two breeds with fewest registrations, Berkshire and Landrace, have a higher inbreeding rate and lower effective population sizes; these breeds need more aggressive conservation in order to maintain genetic diversity. This analysis provides a basis for future monitoring of the genetic diversity of pig breeds.
Key Words: Genetic diversity, Swine, Inbreeding

Introduction

Approximately 20% of the world's breeds are reported to be at risk of extinction (FAO, 2007). Blackburn et al. (2003) detailed the contraction of animal genetic resources (**AnGR**) in the U.S. To address the contraction and potential loss of AnGR, the USDA established the National Animal Germplasm Program (**NAGP**) to conserve livestock and aquatic genetic resources (Blackburn, 2004, 2009). Ideally, genetic conservation efforts would capture all available alleles and their combinations in a population. The U.S. swine industry is highly structured and competitive. As a result, breeders employ high selection intensities for economically relevant traits. As a result of selection pressure and associated inbreeding, allele frequencies can be dramatically changed and there is potential for losing alleles that under the present selection and marketing strategies are not important (Falconer and Mackay, 1996). However, by collecting and cryopreserving germplasm samples, alleles and their various combinations can be made available for future use.

In order for the NAGP genebank to capture the genetic diversity available for each species, the genetic diversity and population status of each species and breed must first be established. Measures to establish the population status include inbreeding levels, registration trends, generation intervals, and effective population size. The objective of this study was to establish a baseline for five major U.S. pig breeds.

Materials and Methods

Animal Care and Use Committee approval was not obtained for this study because the data were obtained from an existing database. Pedigree records were obtained from the National Swine Registry (**NSR**) for Duroc, Hampshire, Landrace, and Yorkshire; Berkshire records were obtained with approval from the American Berkshire Association.

For each breed, a complete pedigree was built until all ancestors were unknown using the Animal Breeders Tool-Kit (**ABTK**; Golden et al., 1992) and the AWK programming language (Aho et al., 1988). The ABTK generates a list of animals that appear as both a sire and dam in the pedigree and animals that appear as their own parent. Data corrections were made; if parentage could not be determined, it was converted to unknown. Inbreeding coefficients (**F**) were computed.

Founder animals, defined as having unknown parents, were assigned a generation number of zero. Then, subsequent generation numbers (**g**) were calculated iteratively as:

$$g = 1/2\ (g_s + g_d) + 1,$$

where g_s is the generation number of the sire and g_d is the generation number of the dam (MacKinnon, 2003). Generation number was compared to mean inbreeding, percent of inbred animals, and number of years of registrations.

Regression procedures were performed using SAS (SAS Inst., Cary, NC). Increases in inbreeding per generation (**ΔF**) were calculated by regressing individual inbreeding coefficients on generation number (MacKinnon, 2003).

Effective population size (N_e), defined as the number of individuals that would generate the current level of inbreeding, was computed as:

$$N_e = 1/2\ \Delta F$$

(Falconer and Mackay, 1996). Generation intervals (**GI**) were computed by regressing generation number on birth year (MacKinnon, 2003).

To represent the current population, F frequencies were calculated for animals born 2006 and later. Coefficient of relationships were computed between the top 10% of boars that sired progeny born 2006 and later (VanRaden and Smith, 1999).

Influential males were determined by computing the number of great-grandprogeny (**GGP**) registered and were grouped into ten percentiles.

Results and Discussion

Summary statistics for each breed are shown in Table 1. The year when records started being stored in electronic format for each breed registry varies, but generally started with animals born in 1980. Number of registrations peaked in 1990 for all breeds, except Berkshire, which peaked in 2000. All breeds have declining registration numbers in the current decade.

Number of dams outnumbered number of sires by approximately 4 to 1. The sire count for the highest number of offspring registered for each breed was 481, 3,797, 1,624, 949, and 1,417 while the dam count was 80, 99, 96, 84, and 142 for Berkshire, Duroc, Hampshire, Landrace, and Yorkshire, respectively. The most prolific Duroc male registered more than 38 times more offspring than the most prolific Duroc female.

The Food and Agriculture Organization of the United Nations (**FAO**; 2000) established an N_e of 50 animals as the critical number to be above; however, Meuwissen and Woolliams (1994) suggested a minimum N_e range of 31 to 250 to maintain population fitness. Duroc, Hampshire, and Yorkshire have relatively robust N_e levels. Berkshire and Landrace are lower and therefore may warrant additional attention. Nicolas (1989) recommended a ΔF rate of < 0.005 as satisfactory, while the FAO (2000) recommended a ΔF rate of < 0.01 as a goal. All breeds meet the FAO goal, but Berkshire and Landrace are above the Nicolas suggested rate ($P < 0.0001$). All breeds had a ΔF that was significantly different from each other ($P < 0.0001$). A rapid turnover of generations for all breeds was found, ranging from 1.65 to 2.21 yrs ($P < 0.0001$). The breeds had significantly different GI ($P < 0.0001$).

The mean F for each breed is reported in Table 1; however, since most of those animals are no longer contributing genes to the future generations, this information is of limited use. Knowing the status of the current population is crucial for conservation activities; Figure 1 shows the F frequencies for animals born 2006 and later. Berkshire (44.7%) and Landrace (39.9%) have a higher percentage of animals with F > 0.10 than the other breeds. In contrast, Duroc has 64% of current animals with $F \leq 0.05$ and 88% with $F \leq 0.10$.

The coefficient of relationships between the top 10% of sires producing progeny born 2006 and later were 0.135, 0.083, 0.122, 0.129, and 0.116 for Berskshire, Duroc, Hampshire, Landrace, and Yorkshire, respectively. With the exception of Duroc, the most popular boars for the remaining 4 breeds are, on average, as closely related as cousins.

After 17 generations, the most generations computed to allow for comparison across all breeds, Landrace has the highest mean F of 17.9% (Figure 2). That is every animal being, on average, somewhere between half-siblings and full-siblings. Hampshire has the lowest F (6.8%). After 12

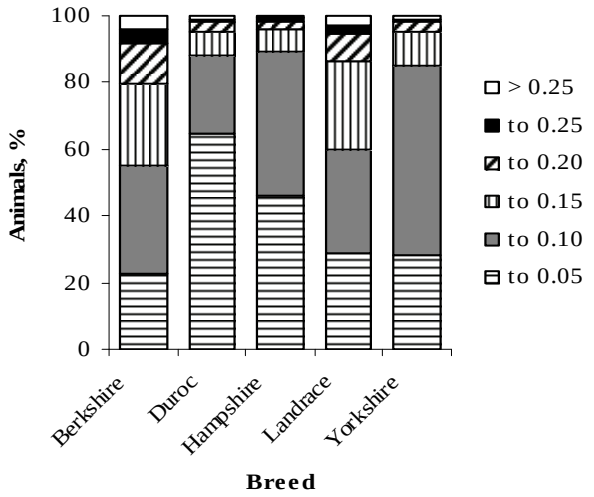

Figure 1. Inbreeding coefficient frequencies by breed for animals born 2006 and later

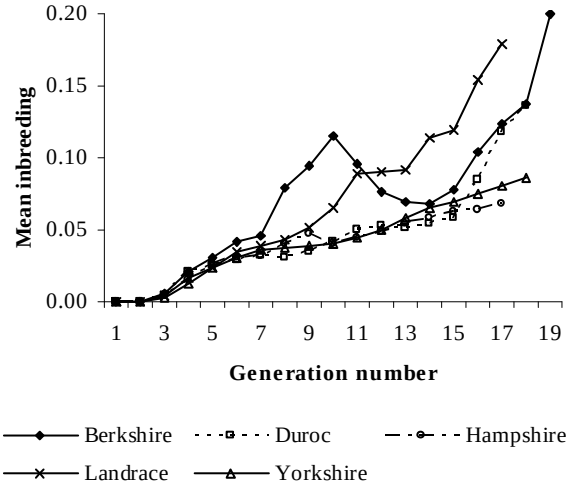

Figure 2. Inbreeding by generation number for all breeds

generations, for all breeds, all animals have an F > 0 (Figure 3). All breeds follow a similar rate of increase in the proportion of inbred animals.

Figure 4 shows how the average generation number increases with number of years of registrations. The

steepest ascent was observed for Berkshire, which also has the shortest GI. Yorkshire plateaus during years 20 to 24, which corresponds to 1992 to 1996. Upon investigation, it was determined there were 13 imported animals, or sons of imported animals, that were contributing between 216 and 1,019 offspring per boar during this time period. To verify if these animals were reducing the slope of the curve, they were assigned the average generation number for their birth year, and the population's generation numbers were recalculated. The slope of the curve increased (Figure 5), showing how influential a few heavily used imported males were for the Yorkshire breed.

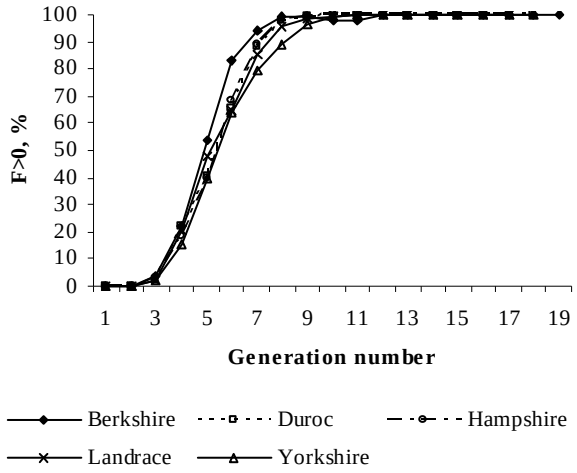

Figure 3. Percent of animals with F > 0 by generation number for all breeds

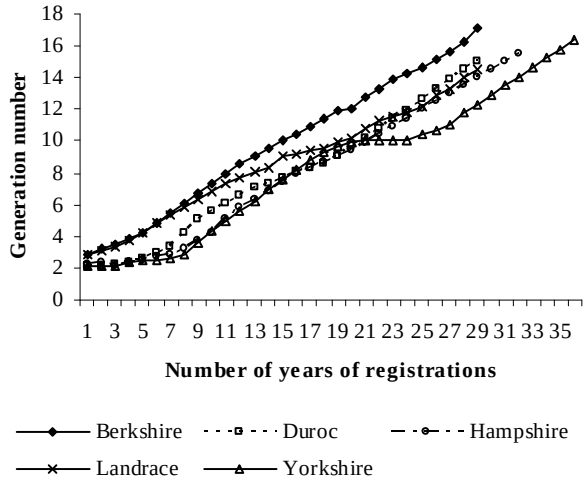

Figure 4. Generation number by number of years of registrations for all breeds

The top 10% of males produced more than 75% of all GGP for all breeds; in Duroc and Hampshire this was more pronounced (85 and 87%, respectively). The bottom 60% of sires produced less than 1% of GGP across breeds. Considering how few males are selected to become sires, and even fewer of those selected males are producing the vast majority of GGP, demonstrates how quickly the genetic base can narrow in a few generations.

With the increase in AI in the swine industry (Blackburn et al., 2003), it will be important to ensure inbreeding levels do not increase more rapidly than they currently are; therefore, the swine industry may wish to incorporate approaches into their genetic evaluation programs that minimize the rate of inbreeding (Meuwissen, 1997).

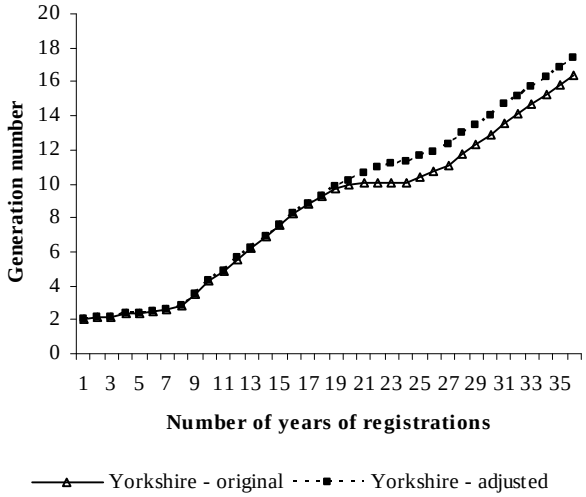

Figure 5. Generation number by number of years of registrations for Yorkshire and for Yorkshire adjusted for 13 sires

Implications

This study serves as a baseline for these five pig breeds in the U.S. Having estimated effective population size, inbreeding levels, and inbreeding rates provides NAGP with greater insight about the genetic diversity of these breeds and how to pursue conservation strategies. While the inbreeding levels are useful genetic diversity indicators, the depth of the pedigrees is relatively shallow given the length of time since these breeds were imported into the U.S. Due to the pedigree depth, it might be safely assumed the reported inbreeding levels are underestimates. Analysis using DNA markers might further elucidate within breed genetic diversity and the genetic differences among the breeds presented in this study (Vicente et al., 2008).

Berkshire and Landrace are intermediate for both effective population size and increase in inbreeding per generation while Duroc, Hampshire, and Yorkshire are within acceptable levels. Inbreeding in the current population is high for Berkshire and Landrace. These two breeds have the fewest registrations, making an even greater challenge for breeders to make mating decisions that minimize long-term inbreeding and maximize performance.

The current trends suggest inbreeding will continue to increase, potentially resulting in loss of alleles from these populations. Broad sampling of lowly related animals within each breed by NAGP is vital to maximize genetic diversity for conservation activities. To date, germplasm collections have been initiated on all five breeds (Table 1); however, additional collections are needed and planned. With the completion of the germplasm collection for each of these breeds, a greater level of protection will be afforded the swine industry.

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Table 1. Summary statistics for pedigree file size, inbreeding (F), sires and dams, change in inbreeding per generation (ΔF), generation interval (GI), effective population size (N_e), and boars in repository for all breeds

Item	Berkshire	Duroc	Hampshire	Landrace	Yorkshire
Pedigree file size	116,758	878,480	744,270	126,566	727,268
Mean F	0.078	0.032	0.036	0.053	0.039
F range	0 to 0.61	0 to 0.58	0 to 0.59	0 to 0.51	0 to 0.65
Unknown sires, %	1.27	1.15	1.04	2.37	3.23
Unknown dams, %	1.26	1.13	1.02	2.38	3.44
Unique sires	6,748	26,615	23,206	7,370	40,458
Unique dams	27,487	126,289	100,246	28,827	175,985
ΔF	0.00647[a]	0.00442[b]	0.00458[c]	0.00674[d]	0.00443[e]
GI, yr	1.65[a]	1.92[b]	2.06[c]	1.83[d]	2.21[e]
N_e	77.28	113.12	109.17	74.18	112.87
Boars in repository, n	29	50	33	29	91

[a-e]Within a row, values without a common superscript differ ($P < 0.0001$).

Table 1. Summary of data for analyses of mature cow weight (MWT, kg) and mature cow height (MHT, cm) for two samples of Angus cows

	Sample 1		Sample 2	
	MWT1	MHT1	MWT2	MHT2
No. Records	23,658	13,012	23,698	13,310
No. Cows	14,056	8,131	15,038	8,439
No. Cont. Groups	1,180	581	1,227	692
No. Pedigree	43,105	43,105	44,141	44,141
Means	596.6	135.7	588.3	134.3

Table 2. Estimates of genetic parameters (SD) for mature cow weight (MWT, kg) and mature cow height (MHT, cm) for two samples of Angus cows (single trait analyses)

	Sample 1		Sample 2	
Estimates	MWT1	MHT1	MWT2	MHT2
Heritability[a]	0.45 (0.012)	0.64 (0.018)	0.48 (0.011)	0.62 (0.018)
Repeatability[a]	0.64	0.77	0.66	0.70
Cont. Group[b]	0.50	0.52	0.52	0.46
Phenotypic Variance	5012.78	36.27	5332.92	33.02

[a] fraction of phenotypic variance not including contemporary group variance.

[b] fraction of phenotypic variance including contemporary group variance.

Table 3. Estimates of genetic parameters for mature cow weight (MWT, kg) and mature cow height (MHT, cm) for two samples of Angus cows (two trait analyses).

	Sample 1		Sample 2	
Estimates	MWT1	MHT1	MWT2	MHT2
Heritability[a]	0.44	0.62	0.47	0.62
Repeatibility[a]	0.64	0.76	0.66	0.70
Cont. Group[b]	0.50	0.53	0.52	0.46
Phenotypic Variance	5009.21	36.08	5285.49	32.65

[a] fraction of phenotypic variance not including contemporary group variance.

[b] fraction of phenotypic variance including contemporary group variance.

Table 4. Estimates of correlations between mature cow weight (MWT) and mature cow height (MHT).

	Sample 1		Sample 2	
	Genetic	Permanent Environmental	Genetic	Permanent environmental
Correlations	0.80	0.75	0.83	0.69

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ESTIMATES OF GENETIC PARAMETERS FOR GROWTH TRAITS OF BRAHMAN, BRANGUS, CHAROLAIS, GELBVIEH AND SIMMENTAL.

A. **Pérez**[1], **M. Montaño**[1] **J. S. Saucedo**[1], **J. A. Terriquez**[1] **J.N. Guerrero**[2]

[1]***Universidad Autónoma de Baja California, Instituto de Ciencias Agrícolas, Avenida Obregón y Julián Carrillo S/N, Mexicali, B.C. México. CP 21100.*** [2] ***University of California, Agricultural and Natural Resources, Imperial County1050 E. Holton Road.***

ABSTRACT. The objective of this study was to estimate genetic parameters of heritability for birth weight BW, weaning weight WW, and yearling weight YW, in a commercial herd located, in Ojos Negros, Baja California, México. It was used the progeny (n=21, n=30, n=33, n=28, and n=32) of heifers and cows of inheritance Brahman B, Brangus Br, Charolais C, Gelbvieh G, and Simmental S, mated to sires (n=3, n=3, n=3, n=3, and n= 4) for B, Br, C, G, and S, respectively. Each trait was analyzed separately by using mixed model methodology. The analytical model included: year of birth, age of cow, sex of the calf, birth date as a covariable to adjust a common age as fixed effects**;** sire and the residual as random components. The (BW), (WW), and (YW) values (34.67±1.06, 37.82±2.51, 41.50± 2.51, 44.42±1.0, and, 39.83±5.1**;** 174.80±8.52, 206.96±29.30, 216.29±18.65, 225.29±17.87, and 266±8.92**;** 239.97±40.88, 261.98±20.24, 268.30±9.57, 298.50±40.16, and 308.19±29.75 kg) corresponded to the progeny of dams involving inheritance of B, Br, C, G, and S, mated to sires to sires involving inheritance of B, Br, C, G, and S, respectively. The estimates of heritability values (h^{2}^= 0.31±0.55, h^{2}^=0.21±0.45, and h^{2}^=0.43±0.63), through the correlation among paternal half sibs corresponded to (BW), (WW), and (YW), respectively.

Key Words: Genetic parameters, Birth weight, Weaning weigth, Yearling weight

Introduction

The key to achieving genetic improvement is that the value of all the positive changes outweigh the value of all the negative changes (Garrick, 2006). This involves identifying animals with the best breeding values, and selecting them to become parents for the next generation (Bichard, 2002). Calf birth weight is positively correlated with weaning, yearling and mature weights. Therefore selection for any of these traits would cause some increase in (BW). However selection only for (BW) will lead to lighter postnatal weight (Bennet et al., 2001). Schemes for changing or limiting changing have been proposed by (MacNeil et al., 1988). The objective of this study was to estimate genetic parameters for BW, WW, and YW, in a commercial beef herd.

Materials and Methods

Progeny (n=21, n=30, n=33, n=28, and n=32) of heifers and cows of inheritance Brahman B, Brangus Br, Charolais C, Gelbvieh G, and Simmental S, mated to sires (n=3 B, n=3 Br, n=3 C, n=3 n=4, and S), respectively. The traits analyzed were: BW (n=144), WW 205 days (n=140), and YW (n=138). Cows were mated artificially AI to produce calves through 7 years of age.

Statistical analyses

Each trait was analyzed separately by using least squares mixed model methodology. The analytical model included: year of birth, age of cow, sex of the calf, birth date as a covariable to adjust a common age as fixed effects**;** sire and the residual as random components.

Results and Discussion

Table 1 shows estimates of mean values (34.67±1.06, 37.82±2.51, 41.50± 2.51, 44.42±1.0, and, 39.83±5.1**;** 174.80±8.52, 206.96±29.30, 216.29±18.65, 225.29±17.87, and 266±8.92**;** 239.97±40.88,

261.98±20.24, 268.30±9.57, 298.50±40.16, and 308.19±29.75 kg) for BW, WW, and YW corresponded to the progeny of dams involving inheritance of B, Br, C, G, and S, mated to sires involving inheritance of B, Br, C, G, and S, respectively.

Birth weight

Kennedy et al. (1971) in a 4 year period study found that BW values (29.70, 31.00 and 31.80 kg) corresponded to calves Brahman, Africander and Shorthorn sired by Hereford, respectively, also indicated that BW was significantly ($P < .01$) affected by age of dams or time of birth (b=0.110 kg per day) but the ranking of the breeds was not altered when the effect of time was removed. Strhobehn et al. (1993) suggests that a program of selection for low BW could lead to declines in WW and YW, which does not seem desirable. Nevertheless in the 1981 Angus sire evaluation report of 673 sires listed, 59 had below average BW but were above average on weaning weight, yearling weight, and maternal breeding value (Strhobehn et al., 1993).

Weaning weight

MacNeil et al. (1982) analyzed (n=47, 652) calf WW records in 371 contemporary groups. Only records on calves with uniquely identified sire and breed dam were used. These authors obtained best linear unbiased estimates (BLUE) for individual and maternal effects on 205-d weights. The authors found that breed effects (± standard errors) were (-22.6 ± 1.3, -19.0 ± 1.1, 12.2 ± 0.9, -11.5 ± 3.1, -10.1 ± 2.2, -7.5 ± 3.41, 5.3 ± 1.6, 9.5 ± 1.8, 11.4 ± 3.1, 12.4 ± 1.2, 14.6 ± 1.2, and 29.7 ± 3.1), for Red Angus (RA), Hereford (H), Angus (A), Polled Hereford (PH), Shorthorn (SH), Tarentaise (T), Gelbvieh (G), Limousin (L), Chianina (CH), Charolais (C), Simmental (S), and Maine Anjou (MA), respectively. The effect of (MA) was ($P<.01$) than that of any other breed. The effect of (L) was less ($P<.01$) than (S), but similar to(CH) and (C). The effect of (G) was significantly less than those of (L), (S), (CH), and (C). Maternal effect of (S) was greater than that of any other breed. Breeds that exhibited individual effects would be more suitable as sire breeds in production systems designated to maximize 205-d WW. Breeds with high maternal effects would be most useful as female parents (MacNeil et al., 1982).

Yearling weight

Gregory et al. (1992) estimated 368-day weight 448 kg in males as overall mean for YW involving inheritance of Red Poll RP, Hereford H, Angus A, Limousin L, Braunnvieh B, Gelbvieh G, Simmental S, and Charolais C. The YW values (410, 382.72, 400.90, 414.09, 463.18, 472.27, 470.90, 478.18, and 470 kg) for RP, H, A, L, B, P, G, S and C), respectively. Based in (n=7055) these authors estimated a mean weighted heritability value (h^2=0.43±0.05) for YW and a phenotypic standard deviation= 76.2 for this trait. The estimates of heritability direct (h^2= 0.43±0.63) at this study was close similar if compared to estimates of (h^2=0.43) found by these authors. However different to estimates of heritability (h^2= 0.33) reported by (Koots et al., 1994 and Green, 1999).

Genetic parameters

Estimates of genetic parameters of heritability (direct) at this study presented in Table 2. As shown The estimates of heritability values and their standard errors were (h^2=0.31±0.55, h^2=0.21±0.45, and h^2=0.43±0.63). These estimates that were calculated through the correlation among paternal half sibs corresponded to (BW), (WW), and (YW), respectively. It can be observed that our estimates of heritability direct (h^2=0.31±0.55 and h^2=0.21 ±0.45) for BW and WW are quite similar to the estimated heritability values by (Koots et al., 1994 and Green, 1999) these authors summarized estimates of heritability direct and maternal (h^2=0.30**;** h^2 =0.14**;** h^2=0.24**;** 0.13, and h^2= 0.33**;** 0.06) for BW, WW, and YW, respectively due to direct and maternal effects; these estimates of h^2 of weighted mean values were based on (n=167, n= 34; 234, n=38, and n= 147, and n=6) research studies for BW, WW, and YW for direct and maternal effects, respectively. However our estimates of h^2= 0.43±0.63 (direct) for YW was different (30.30%) higher compared to the estimated h^2 direct value (h^2= 0.33) of (Koots et al., 1994 and Green, 1999). This difference in magnitude of the estimates could

be attributable to the large number data sets (n=147) used by the authors to arrive to the weighted mean value (h^2=0.33) direct for YW.

Implications

These results show large differences among parental breeds for economically important traits: birth weight, weaning weight and yearling weights. Highest birth weight involved inheritance of Gelvbieh, Charolais, and Simmental. Lowest birth weights involved Brahman and Brangus inheritance. Those differences can be used for breeders in their breeding plans. The additive genetic (direct) effects are also important to achieve a reasonable genetic improvement on those traits.

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Strhoben, D. 1993. The influence of Angus sire birth weight expected progeny difference on the performance of crossbred first-calf heifers. Beef and sheep; a progress report. Iowa State University.

Table 1. Least squares means for birth weight, weaning weight, and yearling weight of progeny of heifers and cows of inheritance Brahman, Brangus, Charolais, Gelbvieh, and Simmental, mated to sires Brahman, Brangus, Charolais, Gelbvieh, and Simmental, respectively.

Breed	Birth weight (kg)	Weaning weight (kg)	Yearling weight (kg)
Brahman	34.67± 1.06	174.80± 8.52	239.97± 40.88
Brangus	37.82± 2.51	206.96± 29.30	261.98 ± 20.24
Charolais	41.50± 2.51	216.29±18.65	268.30± 9.57
Gelbvieh	44.42± 1.00	225.29±17.87	298.50± 40.16
Simmental	39.83± 5.10	266.00± 8.92	308.19± 29.75

Table 2. Heritability (direct) and their standard errors of birth weight, weaning, and yearling weight of progeny of heifers and cows of inheritance Brahman, Brangus, Charolais, Gelbvieh, and Simmental, mated to sires Brahman, Brangus, Charolais, Gelbvieh, and Simmental, respectively.

Trait	Heritablity	Standard errors
Birth weight	h^2= 0.31	±0.55
Weaning weight	h^2= 0.21	±0.45
Yearling weight	h^2= 0.43	±0.63

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ASSOCIATIONS OF PROLACTIN RECEPTOR (PRLR) GENOTYPES AND REPRODUCTIVE TRAITS IN PIGS

A. Barreras-Serrano*[1], J.G. Herrera-Haro[2], S. Hori-Oshima[1], C. Lemus-Flores[3], A.L. Kinejara-Espinosa[1], A. González-Aranguré[1], and J.G. Soto-Avila[1]

Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autónoma de Baja California, 21100. Mexicali, Baja California, México[1], Programa Recursos Genéticos y Productividad-Ganadería, Colegio de Postgraduados, 56100. Montecillo, Edo. de México, México[2], Universidad Autónoma de Nayarit, 63190. Tepic, Nayarit, México[3]

ABSTRACT: The aim of this study was to determine associations between genotypes for the prolactin receptor (PRLR) gene and swine reproductive traits. 329 sows of three genetic groups: Yorkshire (Y), Landrace (L), and YL were included. Reproductive traits studied were: total number of born (TNB), number born alive (NBA), number of weaned piglets (NWP), litter weight at birth (LWB), and litter weight at weaning (LWW). The polymorphism was detected using the polymerase chain reaction–restriction fragment-length polymorphism (PCR-RFLP) method. The association between PRLR genotypes with reproductive traits was evaluated by a linear model. Least square means for all variables were calculated for each genotype in addition to an analysis of additive and dominance effects by genetic groups. Variations due to genetic groups in the frequency of A allele was observed ($P<0.05$). AB genotype in Y breed had the highest values for TNB and different ($P<0.05$) of L. YL showed the best performance for NBA and different ($P<0.05$) of Y. Not difference among genotypes by genetic groups was observed for NWP ($P>0.05$). Differences ($P<0.05$) between genotypes for TNB in first parity with highest value in BB (>10 piglets), were observed in this study. In general, additive effect per allele B resulted in an increase ($P<0.05$) of 2.26 pigs (TNB), and of 0.42 kg (LWB) per litter. Dominance effect was important ($P<0.05$) for TNB and LWB. Additional data is needed to confirm the significant effects of this gene on reproductive traits in swine.

Key Words: Prolactin receptor gene, Litter Size Reproduction, Pigs

Introduction

Reproductive performance determines the economic efficiency in pig production systems because of its effects on productivity. Prolactin receptor (PRLR) is the specific receptor for prolactin, which is an anterior pituitary peptide hormone involved in many different endocrine activities and is essential for reproductive success (Vincent et al., 1998). All actions of prolactin are mediated by its receptor (Van Rens et al., 2003). The prolactin receptor, encoded by PRLR gene, is a member of the growth hormone/prolactin receptor gene family containing regions of identical sequences (Kelly et al., 1991). The prolactin and growth hormone receptors are homologous to receptors for members of the cytokine superfamily (Clevenger et al., 1998). Swine ovaries and endometrium contain PRLRs, which are distributed in a pregnancy-dependent way (Young et al., 1989). Endometrial prolactin receptor numbers increase on day 12 of pregnancy. The increase is stimulated by conceptus estrogen production, which allows for redirection of prostaglandin F2α secretion to support corpus luteum function (Pope, 1994). This implies a potential role of PRLR in preparing and maintaining a proper environment for pregnancy in pigs. Thus, based on the physiological effects, PRLR gene is a strong candidate gene for reproductive traits in pigs.

A positive association was reported between AA genotype and litter size. In first litters, the AA genotype was correlated with higher numbers of piglets born alive (Rothschild et al., 1998; Vincent et al., 1998). Allelic additive effects (a) ranged from zero to 0.59 and 0.71 pigs per litter for total number of piglets born and number born alive, respectively (Vincent et al., 1998). Associations have been reported for Landrace (Vincent et al., 1998).

The aim of this study was to determine associations between genotypes for the prolactin receptor (PRLR) gene and swine reproductive traits.

Materials and Methods

Animals. This study included 329 sows (14 of Landrace (L), 15 of Yorkshire (Y), and 300 of Yorkshire x Landrace (YL) genetic groups) from NW region of México.

DNA samples. A total of 3 ml of blood was collected from each animal in tubes containing a buffer solution of sodium citrate as anticoagulant and used to prepare the package of white blood cells. Whole blood samples were centrifuged at 1000 rpm for 5 min and the supernatant was eliminated. A volume of 5 to 10 ml. of a solution of NaCl at 0.2% was added to the sediment. Then, It was mixed and centrifuged to 2000-2500 rpm for 5 minutes. White cells were recovered as a package and were washed using NaCl at 0.2%. The package was stored at -20ºC. The extraction of DNA was done manually from whole blood using a kit (Ultra Cleanz™ DNA Blood Spin Kit, MO BIO Laboratories, Inc.). For the extraction of DNA a volume of 10 µl of a lysis buffer (100 mM Tris-HCl, pH 7.6, EDTA 40 mM, pH 8.0, 0.5% SDS) was added, followed by a volume of 1/200 of proteinase K 20 $mgml^{-1}$ being incubated at 37ºC from 2 hr to overnight. After 1 or 2 steps extraction of phenol (diluted solution with a buffer TE) and 1 step extraction $CHCl_3$, a volume of 2 of EtOH was added to obtain a precipitate containing

DNA, then DNA was washed with EtOH 75% and re-suspended in sterile distilled water or solution TE buffered for storage at -20ºC.

Genotyping. The genotypes of PRLR gene were identified by means of the PCR-RFLP method. The polymerase chain reaction (PCR) was carried out in 0.2 ml tubes utilizing thermocycler iCycler (Bio-Rad) with primers whose sequences were proposed by Linville et al. (2001). Primers were as follows: the forward primer: 5' CGG CCG CAG AAT CCT GCT GC 3' and the reverse primer: 5' ACC CCA CCT TGT AAC CCA TCA TCC 3. The PCR amplification (25 μL final volume) was performed using 30 ng of genomic porcine DNA, 10× PCR buffer, 2.5 μL each dNTP, 2 μL each primer, and 0.4 μL Taq DNA polymerase (Nova Taq™ DNA). Conditions were 1 cycle at 94°C for 10 min, 40 cycles (94°C, 30 seg; 60°C, 60 seg; and 72°C, 30 seg), followed by 1 cycle at 72°C for 10 seg, stopped to 4°C. After PCR, 5 µl of product was digested by 0.8 μL of restriction enzyme *Alu* I (Fermentas Inc. USA), and the product was resolved in a agarosa gel at 2%. The AB and BB genotypes were distinguishable by the intensity of the 127-bp band, which was much darker in the AB genotype. A monomorphic band of size 35 bp comigrated with the 35-bp digestion product in the B allele.

Reproductive traits. Reproductive traits studied were: total number of born (TNB), number born alive (NBA), number of weaned piglets (NWP), litter weight at birth (LWB), and litter weight at weaning (LWW).

Statistical Analysis. A total of 406 litter records were included in the analyses. The weaning in piglets was reached at 21 days of birth. The association between PRLR genotypes with reproductive traits was evaluated using following linear model:

$$Y_{ijklm} = \mu + G_i + P_j + YS_k + PRLR_l + e_{ijklm}$$

where Y_{ijklm} is the phenotypic record of TNB, NBA, NWP, LWB and LWW, µ is the general mean, G_i is the effect of genetic group of sow (i = L, YL, Y), P_j is the effect of parity number (j = 1, ≥2), YS_k is the effect of the subclass year-season of birth (k = 1.2,…, 8), $PRLR_l$ is the effect of the PRLR genotype (l = AA, AB, BB), and e_{ijklm} is the random error NID (0, σ^2_e). Moreover, all interaction effects were included. Those non-significant interactions (P> 0.10) were not included in the model. The analysis was performed using the GLM procedure in SAS 9.1.3 (Herrera and Barreras, 2005). For additive (a) effect of PRLR genotypes a covariate of the number of favorable alleles in the genotype (0, 1, or 2) while for dominance (d) effect a covariate with values 0,1, and 0 in substitution of AA, AB, and BB genotypes were included in the linear model and estimated utilizing GLM procedure of SAS.

Results and Discussion

The AA genotype represented only 19% in this study. B allele was more abundant in contrast with A allele (0.54 *vs* 0.46), with standard error of .017. Variations due to genetic groups in the frequency of A allele was observed (P<0.05). Frequencies for the A allele were: Landrace = 0.79, Yorkshire = 0.76, and YL = 0.36. Independently of the genetic group, in our study the frequency of PRLR-A allele was higher than the results reported by Hernandez et al. (2006) in México, Kmieć and Terman (2004), and Korwin-Kossakowska et al. (2003) in Poland, but lower than Terman (2005), and similar to Putnová et al. (2002) in Large White pigs. Genotype at the PRLR locus has been shown to explain a significant portion of variation in litter size in Large White, Meishan and Landrace based lines (Vincent et al., 1998). Prolactin affects production of progesterone and relaxin from the corpora lutea (Li et al., 1989).

For the interaction genetic group x PRLR genotype, AB genotype in Y breed had the highest values for TNB and different (P<0.05) of L. YL showed the best performance for NBA and different (P<0.05) of Y. Not difference among genotypes by genetic groups was observed for NWP (P>0.05). Van Rens and van der Lende (2002) conducted a study to determine the effects of PRLR polymorphism on reproductive traits, where the polymorphism at PRLR tended to affect litter size with AA gilts having larger litters. This did not agreed with the result of Drogemuller et al. (2001) where the B allele indicated an additive effect on NBA trait. Isler et al. (2000) also found the B allele to be favorable. They found that it influences significantly the number of fetuses per uterine horn, average fetal weight and total fetal weight in Yorkshire x Large White crossbred pigs. The BB genotype was not found in Landrace and Yorkshire genetic groups in this study. For LWB trait in YL group, BB genotype showed a better (P< 0.01) reproductive performance compared with homozygous AA (15.2 *vs* 14.1 kg, respectively). The results of this study agree partially with Vincent et al. (1998) whose showed that the A allele was significantly associated with increased litter size measured by TNB and NBA. For TNB and LWB traits, in Landrace group, differences between genotypes were observed (P<0.01), with better performance in AA genotype.

Considering that the parents transmit genes and not genotypes to the next generation, is necessary to know the value associated to the gene instead of the genotype, i.e. the average effect of the gene-substitution or additive effect (Falconer and Mackay, 1996). Besides, in populations with the presence of heterozygotes, is important to estimate the interaction value of alleles or dominance effect. A negative increase of 2.26 pigs (TNB), and positive of 0.42 kg (LWB) per litter per copy of allele *Alu*I A and different from zero (P<0.05) was estimated. In the same variables, the dominance effect of PRLR was -2.67 pigs and -0.56 kg, respectively and different (P<0.05) from zero. In general, the additive and dominance effects of alleles for PRLR gene in NBA, NWP, and LWW resulted not different from zero (P> 0.05).

In the analysis by genetic group, Landrace, Yorkshire and YL genetic groups showed additive and dominance effects for TNB, NBA, NWP, and LWB values not different from zero (P> 0.05). For LWW variable, substitution of A for B allele in Landrace group resulted in -8.37 kg (P=0.07) while the value for heterozygosis was de 8.37 kg (P=0.07).

There was no significance (P> 0.05) in additive and dominance effects for LWW in YL and Yorkshire. Drogemuller et al. (2001) reported effects of the A allele ranged from 0.2 piglets per litter difference between homozygotes in Large White to more than one piglet in a Landrace population (Southwood et al., 1995).

Vincent et al. (1998) found inconsistent the mode of additive gene action for allele A on NBA trait with estimates fluctuating from −0.33 to +0.47 piglets per litter. In this study the range of additive effects for NBA was from -0.07 to -0.29 piglets. Furthermore, for Vincent et al. (1998) was not obvious whether PRLR is a major gene for litter size or it is only a linked marker to a gene determining the effect. Associations between the candidate gene and trait may vary between populations, or families. This may be a possible reason for the lack of significant PRLR effects (Drogemuller et al., 2001) or maybe the observed variation among genetic groups could be due only to sampling strategies. One possible reason for the lack of effect in the current study is that different linkage disequilibrium existed in the genetic groups.

Implications

These findings imply that no differences were detected between genotypes for reproductive variables. Independently of genetic group, the additive and dominance effects of alleles for PRLR gene were in TNB and LWB traits, with additive effect in TNB of 2.26 piglets and 0.42 kg for LWB. In the analysis by genetic group, Landrace showed additive effects for LWW values with 8.37 kg. The results suggest a major study of the polymorphism in the PRLR gene and its effects on reproductive traits in order to include the gen information in selection programs.

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Table 1. Least squares means and standard errors for the interaction genetic group x PRLR genotype on reproductive traits[a/] in sows.

genetic group	Genotype	n	TNB	NBA	NWP	LWB	LWW
Landrace	**AA**	27	11.32 ± 0.48 a	9.92 ± 0.47 a	8.92 ± 0.29 ab	14.51 ± 0.66 ab	56.23 ± 2.32 abc
	AB	19	10.34 ± 0.58 b	10.00 ± 0.56 a	9.49 ± 0.34 ab	13.75 ± 0.24 b	64.61 ± 2.76 ab
YL	**AA**	23	10.71 ± 0.53 ab	10.69 ± 0.51 a	8.54 ± 0.30 ab	14.15 ± 0.30 b	59.92 ± 2.51 abc
	AB	169	10.41 ± 0.19 ab	10.42 ± 0.19 a	8.54 ± 0.11 ab	15.26 ± 0.57 ab	57.10 ± 0.92 abc
	BB	108	10.64 ± 0.24 ab	10.65 ± 0.23 a	8.57 ± 0.14 ab	15.21 ± 0.59 ab	57.18 ± 1.16 abc
Yorkshire	**AA**	31	11.22 ± 0.45 a	9.67 ± 0.44 ab	8.38 ± 0.28 ab	15.04 ± 1.60 ab	69.72 ± 8.52 a
	AB	29	11.62 ± 0.47 a	9.55 ± 0.46 ab	8.88 ± 0.28 ab	15.09 ± 0.61 ab	33.00 ± 4.26 d

Least squares means in a column with different letters are statistically different ($P \leq 0.01$)

[a/] TNB = total number of born, NBA = number born alive, NWP= number of weaned piglets, LWB = litter weight at birth, LWW= litter weight at weaning.

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BEEF CALF PERFORMANCE AND CARCASS CHARACTERISTICS FOLLOWING RUMEN-PROTECTED CHOLINE SUPPLEMENTATION OF COWS DURING THE PERIPARTUM PERIOD

J. R. Jaeger[*], K. C. Olson[†], S. R. Goodall[‡], and J. W. Bolte[*]

[*]Western Kansas Agricultural Research Centers, Kansas State University, Hays, KS 67601, [†]Kansas State University, Manhattan, KS 66506, [‡]Feed Industries Consultant, Erie, CO 80516

ABSTRACT: We determined the effects of rumen-protected choline (RPC) supplementation of beef cows during the periparturient period on subsequent calf performance and carcass characteristics at harvest. Angus crossbred cows (n = 181) were stratified by age, BW, and body condition score, and assigned randomly to one of two groups: control (CON) and RPC. Treatments were initiated 50 d before expected onset of calving and continued for 120 d. Cows were maintained in separate groups, and received forage sorghum hay *ad libitum* and 0.91 kg/hd/d ground milo containing a trace mineral supplement; RPC was added to the grain supplement to provide 4 g/hd/d choline for the RPC-treated cows. Calf BW was recorded at birth, weaning, receiving at feedlot, and pre-harvest. All calves received the same finishing diet and were fed to reach an average endpoint of 12 mm of backfat at the 12th rib and harvested in three groups. Calf birth date, BW at birth, 205-d adjusted weaning BW, BW at harvest, and harvest group did not differ ($P > 0.20$) between treatments. Calf ADG from receiving to harvest did not differ ($P > 0.50$) between treatments. Hot carcass weight and dressing percentage did not differ ($P > 0.18$) between treatments and averaged 352.0 ± 3.5 kg and 62.3 ± 0.2%, respectively. Carcass KPH (3.13 ± 0.07%), backfat (0.53 ± 0.02 mm), and marbling score (60.7 ± 1.2) did not differ ($P > 0.19$) between treatments; however, LM area of calves from RPC-supplemented cows was smaller ($P = 0.03$) than the LM area of calves from control cows (75.8 ± 1.3 vs. 80.3 ± 1.2 cm^2, respectively). Yield grade was similar ($P = 0.35$) between treatments and averaged 3.53 ± 0.07; quality grade distribution was similar ($P = 0.62$) between treatments (23.6% Prime, 73.6% Choice, and 2.8% Select). These data were interpreted to suggest that peri-parturient rumen-protected choline supplementation of beef cows had little effect on subsequent performance of calves or their carcass characteristics.

Key Words: Beef Calves, Choline, Supplementation

Introduction

Fetal programming, in theory, results from maternal stimuli during fetal development that influences the physiology of the fetus and postnatal growth and health (Barker et al., 1993). Maternal energy intake of 150 or 215 Kcal ME/kg/d by pregnant beef heifers did not affect nitrogen or gross energy content of fetuses or gravid uteri (Ferrell et al., 1976). Prior and colleagues (1979) concluded that restriction of energy intake by pregnant heifers, when dam BW was maintained, had little influence on fetal development. These authors also reported that plasma NEFA concentrations were not influenced by dietary energy intake. Previously, blood NEFA concentrations were thought to be a useful indicator of nutritional status in ruminant, particularly during pregnancy and lactation.

Increased plasma NEFA results in increased uptake by the liver where NEFA are esterified to triglycerides, oxidized to ketone bodies, or oxidized to carbon dioxide. The esterification of NEFA to triglycerides and their export as VLDL involves choline. In addition, choline serves as a methyl donor for the synthesis of carnitine. Carnitine is essential for fatty acid oxidation. Decreased plasma NEFA of cows supplemented with rumen-protected choline (**RPC**) reported by Pinotti and coworkers (2003) may have resulted from more efficient liver function and improved lipid metabolism. In addition, supplementing beef cows with RPC during the prepartum period tended to increase ADG; however, supplementation during the postpartum period resulted in accelerated weight loss similar to previous reports for dairy cows (Jaeger et al., 2008).

Based on these data, our hypothesis was that supplementation of beef cows with RPC during late gestation may alter plasma NEFA levels without altering energy intake. Therefore, our objective was to determine if providing RPC to beef cows during the final 50 d of gestation could improve subsequent calf performance and carcass characteristics at harvest, possibly through the mechanism of fetal programming.

Materials and Methods

Animals, Treatments and Diet. Procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Angus-cross cows (n = 181; age = 3 to 11 yr) were stratified by age, BW, and body condition score (BCS; 1 = emaciated, 9 = very obese; Wagner et al., 1988) and assigned randomly to one of two treatment groups: control (**CON**) or rumen-protected choline (RPC). Treatments were initiated 50 d before the expected beginning of the calving season and continued for 120 d. During the treatment period, cows were maintained in separate groups and received forage sorghum hay *ad libitum* and 0.95 kg/hd/d supplement. Supplement contained rumen-protected choline (4 g/hd/d choline) and SQM trace mineral (Quali Tech, Chaska, MN) for RPC-treated cows and SQM trace mineral only for control cows

(Table 1). Cows were combined into a single group following the supplementation period.

Table 1. Supplement composition.

	Treatment group	
Ingredient, % DM	Control	RPC
Rolled milo	69.05	69.05
Soybean meal	25.00	25.00
Trace mineral supplement (5.95 %)		
Zinc	0.08	0.08
Manganese	0.08	0.08
Copper	0.03	0.03
Rumen-Protected Choline	0.00	0.54

Calf Management and Data Collection. Treatment effects on early calf performance was assessed by measuring calf BW on the day of birth, when all calves averaged 56 d of age, and at weaning (adjusted to 205 d of age). Upon placement in a feedlot, cattle were weighed and assigned randomly to a receiving pen on the basis of treatment. Calves were adapted to a common receiving diet and fed for 56 d. Calf BW were measured at 28 d intervals during the receiving period. Following the receiving period, replacement heifers were removed from the trial and steers were implanted with Synovex-Choice (Fort Dodge Animal Health, Overland Park, KS). Steers were subsequently adapted to a common finishing diet (Table 2) and fed to a common harvest endpoint. The finishing diet was formulated to achieve an ADG of 1.58 kg at a DMI of 2.5% of BW.

Cattle were fed using a slick-bunk method with feed calls collected each morning before feeding. Daily pen DMI was recorded at 0800 daily. and at 56-d intervals during the finishing phase and also immediately prior to harvest.

Table 2. Average ingredient and nutritional composition of the finishing diet.

Ingredient	%, DM Basis
Ground Sorghum Grain	79.98
Sorghum silage	17.80
Soybean meal	2.08
Limestone	0.25
Ammonium Sulfate	0.11
Nutrient Composition	% of DM
CP	12.84
Ca	0.32
P	0.33
NE_m, Mcal/kg	1.87
NE_g, Mcal/kg	1.24

* Diet also included salt, Rumensin® 80, Tylan® 40, and trace minerals

Harvest and Data Collection. Steers were fed to reach an average endpoint of approximately 12 mm of backfat at the 12th rib and placed into one of three slaughter groups. Once steers reached the targeted carcass endpoint as determined by ultrasound using an Aloka 500V (Aloka Co., Ltd, Wallingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-125 mm window), they were transported 144 km to a commercial abattoir. After the carcasses chilled for approximately 48 h, they were ribbed and graded. Carcass measurements were collected by digital imaging software and included 12th rib fat thickness, 12th rib LM area and marbling score. Using these measurements, yield grade and quality grade were assigned according to USDA (1997). Kidney-pelvic-heart fat was determined by difference in carcass weight after removal of all internal fat by dissection.

Statistics. Calf BW, ADG and carcass characteristics were analyzed as a completely randomized design using the Proc Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Animal served as the experimental unit. Calf DMI and feed efficiency were analyzed using Proc Mixed procedure of SAS with pen serving as the experimental unit.

Results

Average calving date occurred on d 78 of the study. Supplementing dams with RPC had no effect ($P = 0.99$) on calf birth weight which averaged 40.9 ± 2.3 kg. Conversely, calves from CON-supplemented cows tended ($P = 0.08$) to have greater ADG at 56 d of age than calves from RPC-supplemented cows (1.00 ± 0.22 vs. 0.93 ± 0.27 kg/hd/d, respectively). Calf 205-d adjusted weaning BW, BW at harvest, and harvest group were similar ($P > 0.20$) between treatments.

Calf ADG from receiving to harvest did not differ ($P > 0.50$) between treatments. Hot carcass weight and dressing percentage did not differ ($P > 0.18$) between treatments and averaged 352.0 ± 3.5 kg and 62.3 ± 0.2%, respectively (Table 3). Carcass KPH (3.13 ± 0.07%), backfat (0.53 ± 0.02 mm), and marbling score (60.7 ± 1.2) did not differ ($P > 0.19$) between treatments; however, LM area of calves from RPC-supplemented cows was smaller ($P = 0.03$) than the LM area of calves from control cows (75.8 ± 1.2 vs. 80.3 ± 1.3 cm^2, respectively). Yield grade was similar ($P = 0.35$) between treatments and averaged 3.53 ± 0.07; quality grade distribution was also similar ($P = 0.62$) between treatments (23.6% Prime, 73.6% Choice, and 2.8% Select).

Table 3. Carcass characteristics of calves born to dams that received either control (CON) or rumen-protected choline (RPC; 4 g/hd/d choline) supplement for 50 d pre- and 70 d postpartum.

	Treatment group	
Carcass trait	CON	RPC
HCW, kg	355.4 ± 5.4	349.2 ± 4.9
Dressing percentage	62.6 ± 0.3	62.0 ± 0.3
Carcass KPH	3.2 ± 0.1	3.0 ± 0.1
12th-rib fat, cm	1.31 ± 0.06	1.36 ± 0.06
Marbling score	61.9 ± 1.8	59.6 ± 1.6
LM area, cm^2	80.3 ± 1.3	75.8 ± 1.2
Yield grade	3.44 ± 0.13	3.62 ± 0.13
Quality grade, %		
Prime	30.6	17.5
Choice	65.3	80.7
Select	4.1	1.8

Discussion

Zahra and coworkers (2006) reported that periparturient dairy cows with BCS ≥ 4 had greater milk production when supplemented with 14 g RPC/hd/d compared to no RPC. Likewise, Pinotti and coworkers (2003) observed increased milk production following RPC supplementation of periparturient dairy cows with 20 g/hd/d dietary choline. Based on these data, we anticipated an increase in calf ADG during early lactation due to increased milk production; however, we observed that CON calves tended to have greater ADG at 56 d of age compared to RPC calves. Zahra and coworkers (2006) reported that thin cows (BCS < 4) supplemented with RPC and unsupplemented, adequately-conditioned cows produced similar amounts of milk. Cows in our study had an initial average BCS of 5.4 and a final average BCS of 5.3; however, these BCS may not have been adequate to elicit the increased milk production that was previously observed with fleshy dairy cows.

Calves born to dams fed a prepartum protein supplement and calves born to unsupplemented dams had similar BW at birth (Stalker et al., 2006); however, these researchers reported that weaning weight increased due to prepartum supplementation. Larson and colleagues (2009) also reported that weaning weight increased following prepartum protein supplementation of cows grazing native range. Calf BW at birth and weaning were not affected by prepartum supplementation in our study.

Prepartum protein supplementation did not affect feedlot ADG, feedlot DMI, or carcass weight but tended to increase LM area (Stalker et al., 2006; Larson et al., 2009). In addition, prepartum protein supplementation resulted in greater marbling scores,and more favorable USDA quality grade distribution but did not influence USDA yield grades (Larson et al., 2009).

Previous research suggests that fetal programming may occur in winter grazing systems during late gestation when supplemental protein is added to the diet. Increased dietary CP causes increased starch digestion and absorption in conjunction with increased serum insulin concentration (Lopez et al., 2001). In addition, concentrate diets may increase the glucose uptake of intramuscular adipose cells, attributable in part to increased serum insulin (Rhoades et al., 2007). Previous research with dairy cows demonstrated that periparturient RPC supplementation resulted in increased liver glycogen and liver esterified lipids secretion (Piepenbrink and Overton, 2003) and decreased plasma NEFA (Pinotti et al., 2003).

Implications

Supplementation of periparturient beef cows with rumen-protected choline had little effect on subsequent performance of calves or their carcass characteristics.

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POST WEANING MANAGEMENT OF HEIFER CALVES IMPACTS AVERAGE DAILY GAIN AND FEED EFFICIENCY AS PREGNANT HEIFERS

D.M. Larson, T.L. Meyer*, L.A. Stalker and R.N. Funston
West Central Research and Extension Center, North Platte, NE

ABSTRACT: Three experiments evaluated replacement heifer development systems and subsequent effects on gain and efficiency of pregnant heifers. In Exp. 1 and 2, were developed grazing corn residue (**CR**; 422 ± 5 kg) or fed in a dry lot (**DL**; 446 ± 5 kg) prior to breeding. In Exp. 1, a subset of pregnant heifers (n = 40) were individually fed a diet composed of 90% grass hay (11.7 % CP; DM basis) and 10% of a wet distillers grains plus solubles/straw mixture (21.8 % CP; DM basis) during late gestation. In Exp. 2, 55 pregnant heifers that grazed CR (437 ± 8 kg) or were fed in a DL (445 ± 8 kg) prior to breeding, or a mixture of the two (435 ± 8 kg), grazed CR with a supplement (0.45 kg/d; 28% CP) during late gestation. In Exp. 3, 49 pregnant heifers that grazed CR (396 ± 7 kg) or dormant winter range (**WR**; 401 ± 7 kg) prior to breeding, or a mixture of the two (396 ± 8 kg), grazed CR with a supplement (0.45 kg/d; 28% CP) during late gestation. In Exp. 1, pregnant heifers developed in the DL had a greater (P = 0.04) DMI than heifers developed grazing CR, however ADG was not different (P = 0.29). Thus, pregnant heifers developed in the DL had a lower (P = 0.08) G:F than heifers developed grazing CR. In Exp. 2, pregnant heifers grazing CR during late gestation that grazed CR during development gained more (P = 0.04), and maintained a greater (P = 0.08) BCS prior to calving, than heifers developed in the DL. The mixture of CR and DL developed pregnant heifers had an intermediate ADG. In Exp. 3, pregnant heifers grazing CR during late gestation that grazed CR during development gained more (P = 0.02) than heifers that grazed WR or the combination of WR or CR developed heifers. Heifer BCS prior to calving was similar (P = 0.81) in Exp. 3. Heifers grazing CR post weaning gain more and are more efficient while grazing CR as pregnant heifers. The benefit of grazing CR post weaning is most pronounced compared to heifers developed in the DL. These data provide evidence of an adaptive response to grazing low quality forages and may be beneficial in the critical period leading up to the first calving season.

Key Words: Adaptation, Low quality forage, Primiparous heifer

Introduction

Current recommendations indicate a heifer should reach approximately 65% of mature BW by the first insemination for successful reproduction (Patterson et al., 1992). However, recent data demonstrate heifers reaching less than 58% of mature BW by breeding do not display impaired reproductive performance (Funston and Deutscher, 2004; Martin et al., 2008). Heifers developed on an excessively high plane of nutrition have impaired milk production, which reduces productivity (Ferrell et. al., 1976). Heifers developed grazing corn residue (**CR**) gain less during winter grazing, but compensate during the summer, yet are lighter prior to first calving (Larson et al., 2009). Perhaps cows developed grazing CR are more efficient. Lighter cows may have smaller liver mass (Jenkins et al., 1986) and a smaller liver mass is associated with improved feed efficiency (DiCostanzo et al., 1991). There is also anecdotal evidence of a learning curve associated with grazing CR. It may be that cows grazing CR as virgin heifers are better adapted to graze CR prior to calving.

The objective of the current experiments was to evaluate the effect of replacement heifer development system on subsequent gain and efficiency of pregnant heifers.

Materials and Methods

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved the procedures and facilities used in these experiments.

Experiment 1. The effect of heifer development system on ADG and feed efficiency during gestation was evaluated. Following weaning, predominately Angus-based heifers were transported to the West Central Research and Extension Center (**WCREC**), North Platte, NE. After a receiving period, heifers were blocked by initial BW and randomly assigned to graze CR (n = 50) or consume a diet in a dry lot (**DL**; n = 50).

The CR heifers grazed for approximately 88 d and were offered 0.45 kg/d of a 28% CP (DM basis) supplement daily. Following CR grazing, heifers grazed dormant mixed grass upland range with 0.45 kg/d of a 28% CP (DM basis) supplement daily for 60 d. Heifers then entered the DL and were offered a common diet for 47 d until completion of AI. Following weaning, heifers assigned to the DL grazed mixed upland winter range and were offered 0.45 kg/d of a 28% CP (DM basis) supplement daily for 45 d. Heifers then entered the DL and were offered a common diet for 128 d until completion of AI. The DL diet was formulated to achieve an ADG allowing heifers to reach approximately 65% of mature BW (600 kg) prior to AI (NRC, 1996).

Estrus was synchronized using MGA/PGF followed by estrus detection and AI. After AI, heifers were exposed to fertile bulls at a rate of least one bull:50 heifers for 60 d. Approximately 45 d after AI, first service conception was determined via transrectal ultrasonography and final

pregnancy rate was determined via transrectal ultrasonography 45 d after bulls were removed. After pregnancy diagnosis, non-pregnant heifers were sold. During the breeding season and until individual feeding began in October, heifers grazed mixed grass upland summer range in a single group.

Primiparous heifers pregnant by AI (n = 40) were blocked by previous development system and BW. Only heifers pregnant by AI were used to remove variation due to period of gestation. Heifers were originally developed grazing CR (422 ± 5 kg; n = 20) or fed in a DL (446 ± 5 kg; n = 20) prior to first breeding. Heifers were individually fed once daily using a Calan Broadbent feeding system. The heifers were trained to use the system for approximately 25 d prior to the beginning of the 70-d test period. Body weight was measured for three consecutive d at the beginning and end of the study to compute an average. Interim BW was measured every 14 d. The pregnant heifers consumed a diet composed of 90% grass hay (11.7 % CP; DM basis) and 10% of a wet distillers grains plus solubles/straw mixture (21.8 % CP; DM basis) during late gestation. Individual feed offered was recorded daily and individual feed refusal was recorded weekly. Data were analyzed using the MIXED procedure of SAS with the fixed effect of development system and pen as random effect.

Experiment 2. Pregnant heifers grazed CR prior to calving with a supplement (0.45 kg/d; 28% CP) to evaluate effect of heifer development system prior to first breeding on gain during late gestation. Heifers utilized in Exp. 2 were from the same herd as heifers in Exp. 1 and were developed following the same protocols through pregnancy diagnosis. However, heifers used in Exp. 2 were pregnant as a result of a combination of either AI or natural mating.

Pregnant heifers (n = 55) were blocked by BW and mating type and sorted into three groups. The treatment groups included: heifers developed prior to breeding in a DL (445 ± 8 kg; n = 18), heifers developed prior to breeding grazing CR (437 ± 8 kg; n = 18), and a mixture of the two development systems (MIX; 435 ± 8 kg; n = 19). Heifers were transported to CR December 1 and returned to WCREC February 18, grazing CR for 80 d. While grazing CR during late gestation, heifers were offered the equivalent of 0.45 kg/d of a 28% CP (DM basis) supplement provided three times per wk. Heifer BW was measured at d 1, 51 and 80. In addition, heifer BCS was assessed at d 80.

Experiment 3. The effect of development system prior to breeding on gain during late gestation while grazing CR was evaluated. Composite Red Angus × Simmental heifer calves (n = 90) from the Gudmundsen Sandhills Laboratory (**GSL**) near Whitman, NE were assigned randomly by initial BW (225 ± 2 kg) to graze CR or winter range (**WR**) between weaning and the breeding season. Grazing treatments were initiated approximately 30 d after weaning, beginning in mid-November, and continuing through mid-May. Heifers either grazed WR pastures at GSL or were transported to CR fields and grazed for 88 d. A daily supplement was offered (0.45 kg/d; 28% CP) while grazing. Subsequently, all heifers grazed WR for 100 d until breeding with a daily supplement (0.45 kg/d; 28 % CP). Estrus was synchronized with a single i.m. injection of $PGF_{2\alpha}$ administered 108 hr after bulls were turned in with the heifers. Heifers were exposed to fertile bulls (1 bull:25 heifers) for 45 d. Pregnancy diagnosis was performed via transrectal ultrasonography approximately 45 d following completion of the breeding season. After pregnancy diagnosis, non-pregnant heifers were sold. During the breeding season and until grazing CR, heifers grazed upland Sandhills range.

A subset of the pregnant heifers (n = 49) were blocked by BW and sorted into three groups: heifers developed prior to breeding grazing WR (401 ± 7 kg; n = 17), heifers developed prior to breeding grazing CR (396 ± 7 kg; n = 17), and a mixture of the two development systems (MIX; 396 ± 8 kg; n = 15). Pregnant heifers grazed CR during late gestation with a supplement (0.45 kg/d; 28% CP) provided three times per wk in late gestation. Heifers were transported to CR fields December 1 and returned to GSL February 18, grazing CR for 80 d. Heifer BW was measured at d 1, 51, and 80. In addition, heifer BCS was assessed at d 80.

Statistical analysis (Exp. 2 and 3). The corn residue fields were of differing acreage and corn yield. According to the data of Wilson et al. (2004), corn yield influences the carrying capacity of a corn residue field. The relationship between yield and carrying capacity is mass of leaf and husk per acre = ([bushels/acre corn yield x 38.2] + 429) x 0.39. Assuming the forage mass to support 1 AUM is equal to 311 kg of biomass and a 50% utilization rate, then the carrying capacity of a corn residue field may be calculated. The number of AU represented by each individual heifer and the number of AUM supported by the acreage of the field was utilized to adjust the gain data. Subsequently, data were analyzed with MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effects of previous winter development treatment and AUM per field per animal.

Results and Discussion

Heifer gain data for Exp. 1 is summarized in Table 1. In Exp. 1, pregnant heifers developed prior to breeding in the DL had a greater (P = 0.04) DMI than heifers developed grazing CR, however ADG was not different (P = 0.29). Thus, pregnant heifers developed in the DL had a lower (P = 0.08) G:F than heifers developed grazing CR. Previous data indicated heifers developed to a greater weight prior to breeding had a greater liver mass at 72 months of age (Arnett et al., 1971). DiCostanzo et al. (1991) found that cows with a greater liver mass consumed more DM, and were less efficient, than cows with less liver mass. Heifers developed grazing CR were lighter prior to calving than heifers developed in the DL (Larson et al., 2009). Perhaps these lower BW heifers were more efficient due to differences in metabolism. The CR developed heifers may also have experienced compensatory gain, linked to alterations in metabolic hormones such as IGF-1 and T3/T4 (Yambayamba et al., 1996).

Heifer gain data for Exp. 2 is summarized in Table 2. Pregnant heifers grazing CR during late gestation that grazed CR during development gained more (P = 0.04), and

tended to maintain a greater (P = 0.08) BCS prior to calving, than heifers developed in the DL. The mixture of CR and DL developed pregnant heifers had an intermediate ADG but were not different from CR or DL. Heifer gain data for Exp. 3 is summarized in Table 3. In Exp. 3, pregnant heifers grazing CR during late gestation that grazed CR during development gained more (P = 0.02) than heifers that grazed WR or the combination of WR or CR developed heifers. Heifer BCS prior to calving was similar (P = 0.81) in Exp. 3.

Heifers that previously grazed CR were more efficient (DiConstanzo et al., 1991) or experienced more compensatory gain (Yambayamba et al., 1996) than heifers developed in the DL. Heifers developed grazing CR also gained more than heifers developed grazing WR, although precalving BW was not different (Larson et al., 2009). It seems likely a mechanism other than a change in efficiency is partially responsible for the difference in gain.

Previous data has suggested cattle require an acclimation period to grazing corn residue. Research conducted by Fernandez-Rivera and Klopfenstein (1989 a and b) determined that naïve cattle require a learning period when grazing corn residue. Dietary starch content indicated younger cattle consumed less starch in the first 3 wks of grazing compared to older, experienced cattle (Fernandez-Rivera and Klopfenstein, 1989a). Thus, naïve cattle gained less weight early in the grazing season and may lose weight early in the grazing season (Fernandez-Rivera and Klopfenstein, 1989b). Perhaps heifers originally grazing CR during development were better prepared to graze as pregnant heifers, leading to selection of higher quality nutrients and greater gain. Moreover, heifers developed in the DL, grazing CR during the first pregnancy combined with heifers developed grazing CR, gained more than DL developed heifers grazing separately. Although heifers developed grazing CR had a greater BCS prior to calving than heifers developed in the DL, there was no precalving BCS difference between WR and CR developed heifers. Thus, it appears exposing heifers to low quality forage during development better prepares them for grazing CR during the first pregnancy.

Implications

These data provide evidence of an adaptive response to grazing low quality forages and may be beneficial in the critical period leading up to the first calving season. Not only does grazing CR during development improve feed efficiency, but also prepares heifers for grazing CR during pregnancy. Grazing low quality forage during development may produce a heifer better adapted to a lifelong grazing system.

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Table 1. Effect of heifer development system on ADG and feed efficiency of pregnant heifers, Exp. 1

	Treatment[1]			
	DL	CR	SEM	*P*-value
n	20	20		
Initial BW, kg	446	422	5	0.002
Final BW, kg	500	480	6	0.03
DMI, kg	11.7	11.1	0.3	0.04
ADG, kg/d	0.75	0.81	0.04	0.29
G:F, g/ kg	0.065	0.073	0.0	0.08

[1] DL = heifers developed in a dry lot; CR = heifers developed on corn residue.

Table 2. Effect of heifer development system on ADG of pregnant heifers grazing CR, Exp. 2

	Treatment[1]				
	DL	CR	MIX	SEM	*P*-value
n	18	18	19		
Initial BW, kg	445	437	435	8	0.71
Final BW, kg	466	486	469	9	0.27
ADG, kg/d	0.31^{x}	0.58^{y}	0.44^{xy}	0.07	0.04
BCS	5.14	5.47	5.47	0.14	0.08

[1] DL = heifers developed in a dry lot; CR = heifers developed on corn residue; MIX = mixture of heifers from DL and CR treatments.

[xy] Means without a common superscript differ ($P \leq 0.05$).

Table 3. Effect of heifer development system on ADG of pregnant heifers grazing CR, Exp. 3

	Treatment[1]				
	WR	CR	MIX	SEM	*P*-value
n	17	17	15		
Initial BW, kg	401	396	396	8	0.86
Final BW, kg	434	442	429	8	0.54
ADG, kg/d	0.41^{x}	0.60^{y}	0.43^{x}	0.05	0.02
BCS	5.2	5.27	5.18	0.10	0.81

[1] WR = heifers developed on winter range; CR = heifers developed on corn residue; MIX = mixture of heifers from WR and CR treatments.

[xy] Means without a common superscript differ ($P \leq 0.05$).

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EXTENDING GRAZING IN HEIFER DEVELOPMENT SYSTEMS DECREASES COST WITHOUT COMPROMISING PRODUCTION

D.M. Larson[1*], A.S. Cupp[2] and R.N. Funston[1]
[1]West Central Research and Extension Center, North Platte, NE and [2]University of Nebraska, Lincoln

ABSTRACT: Three experiments compared heifer development systems. In Exp. 1, 299 heifers (253 ± 2 kg) from 3 yr were used to compare dry lot (**DL**) to grazing corn residue (**CR**) post weaning. Heifers in the DL consumed a common diet after weaning for 187 d until breeding. The CR heifers grazed for 145 d with a supplement (0.45 kg/d; 28% CP) and were then fed in the DL until breeding. In Exp. 2, 270 heifers (225 ± 2 kg) in 3 yr grazed Sandhills winter range (**WR**) or CR with a supplement (0.45 kg/d; 28% CP) post weaning. In Exp. 3, 180 heifers (262 ± 3 kg) in 2 yr grazed Eastern Nebraska WR or CR with a supplement (0.45 – 0.90 kg/d; 29% CP) post weaning. The CR heifers had lower ($P < 0.001$) ADG before breeding compared to DL or WR heifers in Exp. 1 and 2, but WR and CR were similar ($P = 0.66$) in Exp. 3. The DL and WR heifers were heavier ($P < 0.003$) than CR at breeding and pregnancy diagnosis in Exp. 1 and 2, but similar ($P = 0.62$) in Exp. 3. The percentage of heifers pubertal at breeding was greater ($P < 0.001$) for DL than CR in Exp. 1, for WR than CR in yr 1 and 2 of Exp. 2 ($P < 0.01$), but similar ($P = 0.36$) in Exp. 3. Pregnancy rate to AI was lower ($P = 0.08$) for CR than DL heifers in Exp. 1, but not different ($P = 0.89$) in Exp. 3. Final pregnancy rate was not affected ($P \geq 0.27$) in Exp. 1, 2 or 3. In Exp. 2,, yr 2, CR heifers required ($P = 0.01$) more calving assistance than WR. Milk production of WR heifers was greater ($P = 0.04$) than CR in Exp. 3. Calf weaning BW, two-year old AI (Exp. 1 and 3) and final pregnancy rates (Exp. 1, 2 and 3) were not different ($P > 0.10$). Development grazing CR reduced cost by $45/pregnancy compared to DL, but cost of WR was similar to CR. Development grazing CR reduces ADG before breeding without sacrificing final pregnancy rate. Development grazing WR increases milk production, but does not increase weaning BW. Grazing CR during heifer development reduces cost compared to DL. Grazing CR or WR is suitable for heifer development at similar cost.

Key Words: Dormant forage, Drylot, Heifer development

Introduction

Current recommendations indicate a heifer should reach approximately 65% of her mature BW by the first insemination for successful reproduction (Patterson et al., 1992). Prompted by rising input costs, there is increasing interest in alternative heifer development systems minimizing the use of harvested feedstuffs in favor of grazing. However, dormant forages are lower in available nutrients and may result in poorer animal performance leading to lower BW at breeding. Recent data indicate heifers reaching less than 58% of mature body weight by breeding have similar reproductive ability as heavier counterparts (Funston and Deutscher, 2004; Martin et al., 2008). Moving heifer development out of the dry lot (**DL**) in favor of grazing standing forage may be more cost effective. As corn production increases, so does the availability of corn residue (**CR**) for grazing. Winter range (**WR**) offers a similar source of standing winter forage for heifer development. The effects of developing virgin heifers using standing winter forage are not well characterized. Therefore, the current studies evaluated the effect of grazing CR compared to DL or WR on first service conception rate, pregnancy rate, and first calf production characteristics.

Materials and Methods

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved the procedures and facilities used in these experiments.

Experiment 1. Two hundred ninety-nine crossbred nulliparous heifers (253 ± 2 kg initial BW) from 3 production yr were utilized to compare traditional post weaning DL development to grazing CR during the same period. The heifers in these experiments were predominately black Angus based and purchased from local producers shortly after weaning from their dams.

Following weaning, heifers were transported to the University of Nebraska West Central Research and Extension Center (**WCREC**), North Platte, NE. After a receiving period, heifers were blocked by initial BW and randomly assigned to graze CR or consume a diet in a DL. Heifers assigned to graze CR were shipped to corn fields on approximately November 15 and returned to WCREC between February and April each yr as dictated by weather conditions. Heifers were offered 0.45 kg/d of a 28% CP (DM basis) supplement daily. Heifers grazed CR for approximately 145 d each yr. Subsequently, heifers were transported back to WCREC, placed in the DL and offered a common diet for 42 d each yr. Heifers assigned to the DL treatment were offered a common diet after the weaning period for 187 d each yr until breeding. The DL diet was formulated to achieve an ADG allowing heifers to reach approximately 65% of mature BW (600 kg) prior to AI.

In yr 1, estrus was synchronized using MGA/PGF followed by timed AI (TAI). In yr 2 and 3, estrus was synchronized using MGA/PGF followed by estrous detection and AI. After AI, heifers were exposed to fertile bulls at a rate of least 1 bull:50 heifers for 45 d. Approximately 45 d after AI, first service conception was

determined via transrectal ultrasonography and final pregnancy rate was determined via transrectal ultrasonography 45 d after bulls were removed.

After pregnancy diagnosis, heifers were managed in one group until calving. During the subsequent winter period, all pregnant heifers grazed CR and were offered the equivalent of 0.45 kg/d of a 28% CP (DM basis) supplement provided 3 times per wk. All heifers were weighed prior to calving and calf birth date, birth BW, dystocia score, and sex were recorded at birth.

After calving, all heifers consumed a DL diet through AI breeding. Approximately 60 d after calving, estrus was synchronized using CIDR/PGF followed by timed AI. After AI, heifers were sold to local producers and grazed common summer pastures until weaning. After being sold, all cows were exposed to fertile bulls for a period not less than 45 d. Approximately 45 d after TAI, first service conception was assessed via transrectal ultrasonography. At weaning, final pregnancy rate was determined via transrectal palpation or ultrasonography and calf BW was collected. The data were analyzed using the MIXED and GLIMMIX procedures of SAS.

Experiment 2. Experiment 2 was conducted using heifers from the Gudmundsen Sandhills Laboratory (**GSL**) near Whitman, NE. Composite Red Angus × Simmental weaned heifer calves (n = 270) were assigned randomly by initial BW (225 ± 2 kg) to graze either CR or WR between weaning and the beginning of the breeding season. Grazing treatments were initiated approximately 30 d after weaning, beginning in mid-November, and continuing through mid May each yr. Heifers either grazed winter range pastures at GSL or were transported to corn residue fields on approximately November 15th and returned to GSL on approximately February 15th each yr. A daily supplement was offered (0.45 kg/hd; 28% CP) while grazing. Subsequently, all heifers grazed WR for 100 d prior to breeding with a daily supplement (0.45 kg/hd; 28 % CP) until breeding. Estrus was synchronized with a single i.m. injection of $PGF_{2\alpha}$ administered 108 h after bulls were turned in with the heifers. Heifers were exposed to fertile bulls (1:25; bull:heifer) for 45 d. Pregnancy diagnosis was performed via transrectal ultrasonography approximately 45 d following completion of the breeding season. During the breeding season and until pregnancy diagnosis, heifers grazed upland summer Sandhills range in a single group. After pregnancy diagnosis, non-pregnant heifers were sold.

In the period between pregnancy diagnosis and calving, pregnant heifers grazed upland Sandhills range during the fall until November 15th and then grazed CR during the winter with a supplement (0.45 kg/d, 28% CP) until February 15th. Approximately 2 wk prior to calving, pregnant heifers were weighed and BW recorded. At calving, calf birth date, birth BW, dystocia score, and sex were recorded. At weaning, cows and calves were weighed and BW was recorded. The data were analyzed using the MIXED and GLIMMIX procedures of SAS.

Experiment 3. Experiment 3 was conducted at the Agricultural Research and Development Center near Mead, NE. Composite MARC III x Red Angus weaned heifer calves (n = 180) were assigned randomly by initial BW (262 ± 3 kg) to graze either CR or WR between weaning and breeding. Grazing treatments were initiated approximately 30 d after weaning, beginning in mid-November, and continuing through mid-February (119 d) each yr. A daily supplement was offered (0.45 – 0.90 kg/d; 29% CP) while grazing. Subsequently, all heifers grazed WR for 100 d prior to breeding with a daily supplement (0.45 kg/hd; 28% CP). In addition to grazing, free choice brome hay (13% CP, 42% ADF; DM basis) was offered as weather conditions dictated.

Estrus was synchronized using two i.m. injections of $PGF_{2\alpha}$ administered 16 and 2 d prior to AI breeding. Following the second $PGF_{2\alpha}$ injection, estrus was detected for at least 5 d. Heifers were inseminated approximately 12 h after estrus was detected. Fourteen d after AI, fertile bulls were turned in with the heifers at a ratio of 1 bull:50 heifers. Bulls remained with the heifers for 45 d. Pregnancy to AI was determined via transrectal ultrasonography approximately 45 d after AI. Final pregnancy rate was determined via transrectal ultrasonography 45 d after bulls were removed.

Following pregnancy diagnosis, pregnant heifers were managed in a single group until calving. During this period, pregnant heifers grazed CR with a daily supplement (1.2 kg/d; 10.5% CP). Two wk prior to calving, pregnant heifer BW was measured. At calving, calf birth date, birth BW, dystocia score, and sex were recorded. Between calving and the time when spring pasture was available for grazing, heifers consumed free choice alfalfa/grass hay with a daily supplement (1.2 kg/d; 10.5% CP; DM basis). Approximately 65 d after calving, milk production was estimated using a weigh-suckle-weigh technique. Cow and calf BW were collected at weaning. Data were analyzed using the MIXED and GLIMMIX procedures of SAS.

Results and Discussion

Heifer gain and reproduction data for Exp. 1, 2 and 3 are summarized in Table 1. Heifers grazing CR gained 0.39 kg/d less ($P < 0.001$) than heifers in the DL in Exp. 1 and 0.10 kg/d less ($P < 0.001$) than heifers grazing WR in Exp. 2 during the winter grazing period. Heifers grazing CR in Exp. 3 gained 0.06 kg/day less ($P = 0.002$) than heifers grazing WR. In Exp. 1 and 2, heifers grazed with minimal hay supplementation; however snow cover necessitated more extensive hay feeding in Exp. 3. The ADG during the entire prebreeding phase reflects hay feeding, where heifers grazing CR gained less ($P < 0.001$) than heifers in the DL or grazing WR in Exp. 1 and 2, respectively. However, prebreeding ADG was not different ($P = 0.66$) in Exp. 3. Prebreeding BW was related to prebreeding ADG, where heifers grazing CR were lighter ($P < 0.001$) prior to breeding compared to heifers in the DL (Exp. 1) or grazing WR (Exp. 2). However, prebreeding BW was similar ($P = 0.62$) in Exp. 3. The CR heifers in Exp. 1 were 56% and DL heifers 65% of mature BW before breeding. In Exp. 2, CR developed heifers were 52% and WR heifers 55% of mature BW at breeding. In Exp. 3, CR and WR heifer were approximately 62 to 63% of mature BW at breeding. A summary of previous data indicated heifers should reach 65% of mature BW before breeding for successful reproduction (Patterson et al., 1992). However, data from

our group (Funston and Deutscher, 2004; Martin et al., 2008) demonstrate pregnancy rate, through 4 yr of age is not reduced by developing heifers to less than 53% of mature BW. Likely, due to decreased prebreeding BW, fewer ($P < 0.001$) heifers grazing CR were pubertal before breeding compared to DL heifers in Exp. 1 and compared to WR heifers in yr 1 and 2 of Exp. 2. However, a similar ($P = 0.36$) percentage of heifers from each treatment were pubertal at AI in Exp. 3.

In Exp. 1, AI pregnancy rate was 10% lower ($P = 0.08$) in CR heifers compared to DL heifers, possibly due to pubertal differences. However, AI pregnancy rate was similar ($P = 0.89$) in Exp. 3. Regardless of percentage of pubertal heifers, final pregnancy rate was similar ($P \geq 0.27$) in Exp. 1, 2, and 3. Genetics may have minimized the negative effect of estrous cycle number on pregnancy rate. Byerly et al. (1987) indicated the first estrous cycle a heifer undergoes is less fertile than the third. Cushman et al. (2007) demonstrated that the number of estrous cycles prior to breeding experienced by the first calf heifer is not related to pregnancy rate.

Prior to calving, the CR heifers were still lighter ($P = 0.01$; Exp. 1) than DL heifers, although precalving BW was not different ($P \geq 0.16$) in Exp. 2 and 3. Although lower prebreeding BW may have reduced AI pregnancy rate, the percentage of heifers that calved in the first 21 d of the season was not different ($P \geq 0.20$) between CR and DL (Exp. 1) or CR and WR (Exp. 2 and 3; Table 2). Similar to the percentage calving early, average calf birth date was also not different ($P \geq 0.13$) in Exp. 1, 2 and 3, as were calf birth BW ($P \geq 0.16$) and the percentage of male calves ($P \geq 0.17$). A primary concern associated with this system is an increase in calving difficulty due to lighter heifers at calving. The percentage of heifers requiring calving assistance was not different ($P \geq 0.15$) in Exp. 1 and 3. However, in yr 2 of Exp. 2, 34% more ($P = 0.01$) CR developed heifers required calving assistance than WR developed heifers.

Pregnancy rate to AI in the second breeding season was similar ($P \geq 0.61$) in Exp. 1 and Exp. 3 (Table 1). Final pregnancy rate after the second breeding season was also similar ($P \geq 0.37$) between treatment groups in Exp. 1, 2 and 3. Apparent milk production was measured in Exp. 3 (Table 2). The WR developed heifers produced more milk ($P = 0.04$) at approximately 65 d post calving than heifers developed grazing CR. However, neither calf weaning BW ($P \geq 0.44$) or calf adjusted 205 d BW ($P \geq 0.31$) were different among treatments in Exp. 1, 2 or 3. These data agree with previous research conducted by Funston and Deutscher (2004) and Martin et al. (2008) who indicate that although heifers developed to 50% of mature BW at breeding are lighter through the third breeding season, long term reproduction and calf production are not impacted.

Previous data (Funston and Deutscher, 2004; Martin et al., 2008) demonstrate substantial cost reductions from lower gain heifer development. These previous studies were conducted with heifers developed in the drylot targeted for lower rates of gain. Thus, developing heifers using dormant, standing forage may further reduce cost. Non-pregnant heifers developed grazing standing forage are lighter at pregnancy diagnosis than traditionally developed heifers and will be better suited for a long-yearling feedlot program. Cull heifers are considered an additional source of revenue in this system. Developing heifers by grazing CR reduced winter feed cost by $42/heifer compared to development in the dry lot (Table 3). In addition, slightly more CR heifers were not pregnant after breeding, increasing the value of culled heifers. After considering feeding cost and cull value difference, CR development reduced the net cost of developing one pregnant heifer by $45 compared to DL development. However, as WR and CR are charged to the development system at a similar cost and pregnancy rates were similar, developing heifers on CR or WR resulted in little difference in the cost of developing a pregnant heifer.

Implications

Winter development using corn residue is a suitable alternative to winter range or a dry lot. The reduction in the percentage of pubertal heifers developed grazing corn residue may reduce AI conception, but final pregnancy rate is similar. The factors that mediate these effects are complex; however, developing heifers using corn residue does not negatively influence long-term production. Developing heifers by grazing dormant forage reduces cost compared to dry lot feeding, improving sustainability.

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Table 1. Effect of winter system on gain and reproduction in heifers, Exp.1, 2 and 3

	Treatment											
	Exp. 1[1]			Exp. 2[2]			Exp. 3[3]			*P*-values		
Item	DL	CR	SEM	WR	CR	SEM	WR	CR	SEM	Exp.1	Exp.2	Exp.3
n	150	149		136	134		90	90				
Pre-breeding BW, kg	387	336	4	298	244	2	313	308	3	<0.001	<0.001	0.62
Percentage of mature BW	65	56	1	55	52	1	63	62	1	<0.001	<0.001	0.62
Pregnancy diagnosis BW, kg	444	416	5	359	349	2	420	416	4	<0.001	0.003	0.44
ADG during grazing, kg/d[4]	0.58	0.19	0.01	0.24	0.14	0.01	0.43	0.37	0.01	<0.001	<0.001	0.002
Prebreeding ADG, kg/d[5]	0.68	0.42	0.01	0.38	0.29	0.01	0.54	0.55	0.01	<0.001	<0.001	0.66
ADG from breeding to pregnancy diagnosis, kg/d	0.47	0.67	0.08	0.67	0.73	0.01	0.46	0.41	0.02	<0.001	<0.001	0.05
Pubertal by AI, %	88	46	4	-	-	-	57	63	5	<0.001	-	0.36
Year 1	-	-	-	73	33	7	-	-	-	-	<0.001	-
Year 2	-	-	-	77	61	8	-	-	-	-	<0.001	-
Year 3	-	-	-	49	58	7	-	-	-	-	0.003	-
Pregnant to AI, %	64	54	8	-	-	-	43	44	5	0.08		0.89
Yearling pregnancy, %	94	92	5	85	84	3	83	89	4	0.37	0.85	0.27
n	88	75		72	75		24	26				
Precalving BW, kg	446	428	5	444	440	4	469	461	4	0.01	0.33	0.16
AI pregnant, 2-year old, %	62	66	6	-	-	-	61	56	10	0.61	-	0.75
Pregnant, 2-year old, %	87	81	5	85	77	7	92	100	6	0.39	0.37	0.98

[1] DL = developed in the dry lot, CR = developed on corn residue (145 d) and fed in the dry lot (42 d) before AI.
[2] WR = developed on winter range, CR = developed grazing corn residue (100 d) and grazed winter range (100 d) before breeding.
[3] WR = developed on winter range, CR = developed grazing corn residue (120 d) and grazed winter range (100 d) before AI.
[4] ADG during the winter grazing period; [5] ADG after the winter grazing period prior to breeding

Table 2. Effect of winter system on calf production, Exp.1, 2 and 3

	Treatment											
	Exp. 1[1]			Exp. 2[2]			Exp. 3[3]			*P*-values[2]		
Item	DL	CR	SEM	WR	CR	SEM	WR	CR	SEM	Exp.1	Exp.2	Exp.3
n	88	75		72	75		24	26				
Calved in 1st 21 d, %	84	76	11	83	75	5	81	74	5	0.20	0.24	0.32
Calf birth date, Julian d	71	74	3	67	69	1	76	75	3	0.13	0.37	0.75
Calf birth BW, kg	35	34	1	32	33	1	33	35	1	0.16	0.35	0.17
Assisted births, %	26	33	5	-	-	-	8	22	8	0.33	-	0.15
Year 1	-	-	-	37	28	8	-	-	-	-	0.40	-
Year 2	-	-	-	13	47	9	-	-	-	-	0.01	-
Sex, % male	55	48	3	49	55	6	84	73	6	0.41	0.43	0.17
Milk production, kg/24 h[4]	-	-	-	-	-	-	4.1	2.9	0.6	-	-	0.04
Calf weaning BW, kg	193	197	5	178	181	4	220	226	5	0.49	0.59	0.44
Calf 205 d BW, kg	180	186	4	195	197	3	215	219	5	0.31	0.59	0.51

[1] DL = developed in the dry lot, CR = developed grazing corn residue (145 d) and fed in the dry lot (42 d) before AI.
[2] WR = developed on winter range, CR = developed grazing corn residue (100 d) and grazed winter range (100 d) before breeding.
[3] WR = developed on winter range, CR = developed grazing corn residue (120 d) and grazed winter range (100 d) before AI.
[4] Measured using a modified weigh-suckle-weigh technique approximately 65 d post calving.

Table 3. Effect of winter system on heifer development cost , Exp.1, 2 and 3

	Treatment								
	Exp. 1[1]			Exp. 2[2]			Exp. 3[3]		
Item	DL	CR	Diff	WR	CR	Diff	WR	CR	Diff
n	150	149		136	134		90	90	
Feeding cost, $/heifer	237	195	-42	124	123	-1	128	121	-8
Total development cost, $/heifer	982	941	-41	832	838	6	853	848	-5
Cull heifer value, $/heifer exposed	53	77	-24	131	135	4	160	104	-56
Net cost of 1 pregnant heifer, $	985	940	-45	821	832	11	831	835	4

[1] DL = developed in the dry lot, CR = developed grazing corn residue (145 d) and fed in the dry lot (42 d) before AI.

[2] WR = developed on winter range, CR = developed grazing corn residue (100 d) and grazed winter range (100 d) before breeding.

[3] developed on winter range, CR = developed grazing corn residue (120 d) and grazed winter range (100 d) before AI.

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ESTROUS SYNCHRONIZATION INCREASES EARLY CALVING FREQUENCY, WHICH ENHANCES STEER PROGENY VALUE

D.M. Larson* and R.N. Funston
West Central Research and Extension Center, North Platte, NE

ABSTRACT: Calving records collected between 2000 and 2008 at the Gudmundsen Sandhills Laboratory, Whitman, NE, were used to determine the effect of estrous synchronization on calving distribution and the impact of time of calving on carcass characteristics. Calves born between 2000 and 2006 resulted from non-synchronized 60 d breeding seasons between 1999 and 2005 (n = 2075). Calves born in 2007 and 2008 resulted from synchronized 45 d breeding seasons in 2006 and 2007 (n = 521). Estrus was synchronized with a single injection of prostaglandin $F_{2\alpha}$ administered 108 h after bulls were turned in with cows. Cow pregnancy rate after synchronized or non-synchronized breeding seasons was similar (P = 0.48). Twelve percent more ($P < 0.001$) synchronized cows calved during the first 21 d compared to non-synchronized cows. Average calving date and percentage of male calves were similar ($P \geq 0.23$). The weaning BW of calves born to synchronized dams was 9 kg greater ($P < 0.001$) than calves from non-synchronized dams. The effect of calving distribution, defined as percentage calving in the 1st, 2nd or 3rd 21 d of the season was evaluated in the steer progeny born between 2001 and 2007 (n = 659). Steers were fed in the feedlot and slaughtered after 218 d on feed. As the time of calving increased, male calf birth weaning BW decreased ($P < 0.001$). Time of calving did not affect feedlot ADG (P = 0.90). As time of calving increased, HCW, marbling score and yield grade decreased ($P < 0.001$). Although the percentage of steers achieving USDA small grade was not affected (P = 0.17) by time of calving, the percentage of steers receiving a USDA quality grade of modest or greater and the total carcass value declined ($P \leq 0.001$) as time of calving increased. Estrous synchronization with a single injection of prostaglandin $F_{2\alpha}$ resulted in more cows giving birth earlier, even though the breeding season was 15 d shorter. Calves born earlier in the season are heavier at weaning and produce a heavier, more valuable carcass.

Key Words: Calving distribution, Carcass quality, Estrous synchronization, Weaning weight

Introduction

Estrous synchronization is utilized primarily in conjunction with artificial insemination. However, estrous synchronization is potentially beneficial to cattle producers using natural mating. A primary obstacle to increased usage of estrous synchronization is the labor associated with applying a synchronization protocol. Thus, a successful system will be easy to implement and cost effective. Prostaglandin $F_{2\alpha}$ (**PGF**) causes lysis of the corpus luteum (**CL**) when administered at least 96 h after ovulation; however, the corpus luteum is not responsive to PGF prior to this time. Standing estrus will occur between 48 and 96 h after PGF in cyclic females. Whittier et al. (1991) demonstrated a single PGF injection administered 96 h after bull turn-in increased the percentage of cows calving in the first 50 d of the calving season. However, they did not detect a difference in the percentage calving in the first 21 d, nor did they measure weaning BW or carcass characteristics of the resulting calf crop. Data from our group (Larson et al., in press) indicate more heifers given PGF 96 h after bull turn-in calved in the first 21 d of the calving season. Further research is needed to evaluate the effect of this system in mature, lactating cows. Thus, data from eight production yr were summarized to determine the effect of estrous synchronization on time of calving and subsequent effects of time of calving on carcass characteristics.

Materials and Methods

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

Breeding, calving, weaning, and carcass data were collected from the research herd at the Gudmundsen Sandhills Laboratory (**GSL**) near Whitman, NE. The data for the spring calving herd, collected between 2000 and 2008, were used for the purposes of this analysis. Calves born between 2000 and 2006 resulted from non-synchronized 60 d breeding seasons between 1999 and 2005 (n = 2075). Calves born in 2007 and 2008 resulted from estrous synchronized 45 d breeding seasons in 2006 and 2007 (n = 521). The exception was a subset of cows used in a nutritional experiment exposed to bulls for 60 d during the estrous synchronized spring breeding season in 2007 (118 cows). The breeding season begins on approximately June 15. Estrus was synchronized using a single injection of PGF administered 108 h after fertile, mixed age bulls were turned in with the cowherd. The bull to cow ratio was at least 1:25 in all years. Pregnancy was diagnosed via rectal palpation approximately 45 d following bull removal. As varying nutritional and breeding treatments are applied to the yearling heifers during breeding, two year-old cows were removed from this analysis to avoid confounding the results. Weaning data were analyzed for the 2007 and 2008 weaned calves (408 individual records) and compared to calves weaned between 2000 and 2006 (1790 individual records).

Weaned steers (n = 659) in each yr were transported to the West Central Research and Extension Center in North Platte, NE. The data from these steers were used to determine the effect of early calving frequency on feedlot performance and carcass quality. Steers were fed a common diet, within yr, in the feedlot for approximately 218 d. Steers were slaughtered at a commercial abattoir when 12th rib fat cover was visually assessed to be approximately 1 cm. Routine carcass data were collected after slaughter. Carcass characteristics were evaluated by period of calf birth the first, second, or third 21 d period of the calving season. The continuous data were analyzed using the MIXED procedure of SAS and binomial data with the GLIMMIX procedure of SAS. The model included the fixed effect of estrous synchronization and the age of the dam. The model also included the random effects of year and any treatments imposed on each particular herd within each year.

Results and Discussion

The data demonstrating the effect of estrous synchronization on reproduction and calf production are displayed in Table 1. The estrous synchronized subset of data was generated for the 2007 and 2008 calving seasons and the non-synchronized subset was generated for the years between 2000 and 2006.

Calf birth date was similar ($P = 0.23$) between estrous synchronized and non-synchronized cows however, calf birth BW ($P < 0.001$) and the incidence of dystocia ($P < 0.001$) were lower if from synchronized dams. The percentage of male calves was unaffected ($P = 0.62$) by estrous synchronization. Perhaps most interesting, estrous synchronization increased ($P < 0.001$) the percentage of cows giving birth in the first 21 d by 12% (73 vs. 61%; estrous synchronized vs. non-synchronized, respectively). This may partially explain the reduction in birth BW. Cows at GSL calve in a common group and consume a higher quality diet during calving than during late gestation. Thus, cows calving later are on a higher plane of nutrition during late gestation than earlier calving cows, perhaps leading to heavier calves at birth. Whittier et al. (1991) found that a single injection of PGF administered 96 h after bull turn-in increased the percentage of cows calving in the first 50 d of the calving season. However, they detected no difference in the percentage calving in the first 21 d. Data from our group indicate more heifers injected with PGF 96 h after bull turn-in calved in the first 21 d of the season (Larson et al., in press).

The mechanism underlying this estrous synchronization system relies on the observation that the CL is unresponsive to PGF within 96 h after ovulation. Thus, bulls are allowed to inseminate cows at natural estrus for approximately 5 d; cows inseminated during this period will not respond to PGF. On d 5, PGF is administered to all cows and the bulls inseminate cows at synchronized estrus following PGF. It is imperative to administer PGF at the correct interval to avoid destroying the CL in cows inseminated on the d of bull turn-in. These data agree with previously published research in both mature cows and replacement heifers (Whittier et al., 1991 and Larson et al., in press). Calf birth date was unaffected, which may seem counterintuitive. Most likely, cows that fail to conceive at the synchronized estrus are inseminated 21 d later and thus average calving date is unaffected. As further evidence, 96 and 94% of the 94 to 95% of cows that became pregnant (estrous synchronized and non-synchronized; respectively), calved within the first 42 d of the season. Regardless, more calves are born early in the season with estrous synchronization. As more calves are born earlier in the season, one may expect weaning BW to be increased. Accordingly, calves from estrous synchronized dams were 9 kg heavier ($P < 0.001$) than calves from non-synchronized dams. This would likely make calves from estrous synchronized dams more valuable at weaning, improving profitability. Although the natural breeding season was shortened when estrous synchronization began, pregnancy rate was unaffected ($P = 0.48$) by synchronization. Perhaps this indicates a more efficient use of bull resources during the breeding season. At pregnancy diagnosis, both cow BW and BCS were similar ($P \geq 0.16$) between estrous synchronized and non-synchronized cows.

Estrous synchronization increased the percentage of cows calving in the first 21 d of the breeding season (Table 1). This indicates more cows were mated by natural service early in the breeding season. Estrous synchronization increased calf weaning BW and potential value. In addition, the breeding season was shortened from 60 to 45 d between non-synchronized and estrous synchronized seasons, respectively without negatively affecting pregnancy rate. In relation to the increased weaning BW associated with early calving, we sought to determine if early calving frequency affected carcass traits.

Recall that estrous synchronized cows, more of whom calved in the first 21 d of the season, gave birth to lighter calves. However, when evaluating only steer progeny, male calves born earlier in the season did not have a lighter ($P = 0.47$) birth BW than those born later. As the time of calving became more advanced, steer weaning BW was lower ($P < 0.001$) with each successive interval, likely related to calf age. Neither preweaning ($P = 0.92$) nor feedlot ADG ($P = 0.90$) were affected by time of calving.

Similar to weaning BW, HCW increased ($P < 0.001$) coordinately with early calving frequency. Perhaps more interestingly, marbling score and the percentage of steers achieving a USDA quality grade of modest or greater was greater ($P = 0.001$) in steers born earlier than those born later. It was, and perhaps still is, a common paradigm that intramuscular fat is a late developing trait. These data would support the hypothesis steers born earlier in the calving season are older at harvest. The increase in marbling score cannot be separated from a difference in caloric intake as DMI was not measured. However, older steers are also fatter, as evidenced by an increase ($P < 0.001$) in yield grade of earlier born steers. As time of calving became more advanced, the percentage of empty body fat ($P < 0.001$) decreased. Thus, it appears as time of calving advances, carcass fat content in all depots, including intramuscular, decreases. Although later born steers had a slightly lower yield grade, the reduction in marbling score made their carcasses less valuable ($P < 0.001$). The difference in carcass value is also related to the

increased HCW of steers born earlier in the calving season. Therefore, carcasses of earlier born steers were more valuable on a weight basis and received a greater premium on a carcass basis than later born steers.

Implications

Estrous synchronization with a single injection of PGF can increase the percentage of cows naturally mated early in the breeding season. This improvement occurs even in a shorter breeding season. Moreover, most cows not mated at the first estrus become pregnant at the second. Steer calves born earlier in the calving season have greater weaning BW, HCW and marbling scores. Improving early calving frequency may increase progeny value at weaning and enhances carcass value of the steers.

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Table 1. Effect of estrous synchronization in a natural mating system on reproduction and calf production

	Estrous synchronized			
Item	No	Yes	SEM	*P*
n	2075	521		
Calf birth date, Julian d	86	85	1	0.23
Calf birth BW, kg	38	37	1	<0.001
Assisted births, %	4.4	1.7	5	<0.001
Calved in 1st 21 d, %	61	73	2	<0.001
Calved in 2nd 21 d, %	33	23	2	<0.001
Calf sex, % male	51	52	2	0.62
n	1790	408		
Calf weaning BW, kg	219	228	3	<0.001
Cow BW at weaning, kg	505	502	4	0.16
Cow BCS at weaning	5.2	5.2	0.1	0.25
Pregnant, %[1]	95	94	1	0.48

[1] Pregnancy rate after an estrous synchronized or unsynchronized natural mating season.

Table 2. Effect of calving period on feedlot performance and carcass characteristics of steer progeny

	Calving period[1]				
Item	1	2	3	SEM	*P*
n	347	259	53		
Calf birth BW, kg	37	37	37	1	0.47
Calf weaning BW, kg	233[a]	219[b]	197[c]	6	<0.001
Preweaning ADG, kg/d	0.96	0.96	0.97	0.02	0.92
Feedlot ADG, kg/d	1.64	1.64	1.65	0.05	0.90
HCW, kg	370[a]	363[b]	350[c]	5	<0.001
Marbling score[2]	574[a]	554[b]	527[c]	15	<0.001
Empty body fat, %	30.4[a]	29.9[b]	29.0[c]	0.4	<0.001
Yield grade	3.0[a]	2.8[b]	2.6[c]	0.2	<0.001
Choice or greater, %	84	83	73	8	0.17
Average choice or greater, %	30[a]	17[b]	12[b]	5	0.001
Carcass value, $	1102[a]	1079[b]	1025[c]	45	<0.001

[1] 1 = calved in the 1st 21 d, 2 = calved in the 2nd 21 d, 3 = calved in the 3rd 21 d.

[2] 500 = small[0].

[abc] Means without a common superscript differ ($P \leq 0.05$).

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INCREASING GLUCOGENIC PRECURSORS IN RANGE SUPPLEMENTS IMPROVES REPRODUCTIVE EFFICIENCY AND PROFITABILITY IN YOUNG POSTPARTUM RANGE COWS IN YEARS 2000 TO 2007

J. T. Mulliniks[1], S. H. Cox[1], M. E. Kemp[1], R. L. Endecott[2], R. C. Waterman[3], D. M. VanLeeuwen[1], and M. K. Petersen[3]

[1]New Mexico State University, Las Cruces, NM
[2]Montana State University, Miles City, MT
[3]USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT

ABSTRACT: Reproductive efficiency in young beef cows is often compromised due to a mismatch of physiological demands and suboptimal environmental conditions. Studies conducted at the Corona Range and Livestock Research Center from 2000 to 2007 evaluated 3 postpartum supplement strategies increasing in glucogenic precursors (**GP**). Reproductive variables, milk production, and serum metabolites were used to assess supplement effectiveness and economics associated with young beef cow production (n = 379) on native range. Supplements were individually fed 2×/wk at 1135 g/d (2003-2004) or 908 g/d (all other yr) and provided: 1) 327 g CP, 109-118 g undegradable intake protein (**UIP**), 44-47 g GP (**CON**); 2) 327-341 g CP, 142-157 g UIP, 57-70 g GP (**BP**); 3) 327 g CP, 151-173 g UIP + 40 – 100 g of propionate salt (NutroCal, Kemin Industries, Inc.), 93-141 g GP (**P**). Blood samples were collected 1×/wk (2000) or 2×/wk (2001-2007) for progesterone analysis to estimate days to first estrus. Cows were exposed to bulls for 60 d or less and pregnancy was confirmed by rectal palpation at weaning. Number of days to first estrus after calving decreased and pregnancy rates increased linearly ($P \leq 0.02$) with increasing supplemental GP. Milk production exhibited a quadratic ($P = 0.04$) response to increasing GP with cows fed BP producing the most amount of milk (5920, 6812, and 6217 ± 421 g/d for CON, BP, and P, respectively). Total kg of calf weaned per cow exposed for the supplemental year and subsequent year was greater ($P = 0.07$) for cows fed P than the other supplements (418, 410, and 435 ± 40 kg for CON, BP, and P, respectively). These data suggest feeding young cows additional GP in the form of propionate salts allows for repartitioning of nutrients away from milk production and towards reproduction.

Key Words: Beef Cow, Supplementation, Reproduction

INTRODUCTION

Achieving reproductive efficiency in a beef cow herd is a critical factor for beef cow/calf producers to be sustainable. Reproduction is the primary cause limiting production efficiency in most herds, in which failure to conceive represents the single most important factor reducing net calf crop (Dziuk and Bellows, 1983). Furthermore, reproduction is associated with five times more economic value than traits associated with milk production or calf growth (Trenkle and Willham, 1977).

In a beef cow herd a lower percentage of successful rebreedings of young cows is a large cost and an obstacle to profitability. One reason for a lower rebreeding rate in young cows is their inability to consume enough energy for maintenance, lactation, and growth due to their immature body weight. Decreased efficiency in young beef cows can be due to reduced forage quality coupled with higher energy demands (Hawkins et al., 2000). Glucose requirements are increased dramatically due to nutrient demands of lactation plus a grazed dormant native range that yields low quantities of glucogenic precursors, specifically ruminal propionate. Therefore, the supply of glucose precursors becomes increasingly important for reproductive competence when forage quality is low (Hawkins et al., 2000). Knox (1998) found that young cows fed protein supplements increasing in glucogenic precursors coming from differing amounts of bypass protein had an 18% increase in pregnancy rates than cows fed a low bypass supplement. Therefore, the objective of this experiment were to compare measures of reproductive efficiency such as pregnancy rate, days to first estrus, and calf weight per cow exposed as impacted by 3 different postpartum supplementation strategies increasing in glucogenic potential and to assess economic viability of these supplementation strategies for 2- and 3-yr-old cows grazing native range.

MATERIALS AND METHODS

Studies were conducted over 8 years at New Mexico State University's Corona Range and Livestock Research Center (CRLRC). The data were compiled from three independent studies: 1) Waterman et al., 2006 (2000-2001), 2) Endecott et al., 2006 (2003-2004), and 3) Mulliniks et al., 2008 (2005-2007). A study did not occur during 2002 because of drought conditions. The ranch's average elevation is 1,900 m with an average rainfall of 380 mm, most of which occurs in July and August (Waterman et al., 2006). Forages at this study site were primarily blue grama (*Bouteloua gracilis*), threeawns (*Aristida* spp.), and wolftail (*Lycurus phleoides*). All animal handling and experimental procedures were in accordance with guidelines set by the New Mexico State University's Institutional Animal Care and Use Committee.

Animals and Supplementation. Cows were 2- and 3-yr-old (n = 379) and were primarily Angus breeding with some Hereford influence. Management before calving was similar in all years and among all cows. Cow/calf pairs

were moved to a common pasture within 10 d of calving. In all years, cows were stratified by calving date and were randomly assigned to treatments. Initiation of supplementation occurred approximately 10 d after parturition. A 60-d breeding season was utilized in all years and was initiated in early or mid May. Cows were moved to an ungrazed pasture prior to the initiation of breeding in all years.

On supplementation days, cows were gathered and calves were sorted off after the morning grazing bout. Strategically, cows were individually fed supplement for an average of 90 d (2000), 100 d (2001), 72 d (2003), 65 d (2004), 74 d (2005), 120 d (2006), and 80 d (2007). Duration of supplementation was dictated by environmental conditions and the onset of BW gain. Supplements were cubed and milled at Hi-Pro Feeds, Friona, TX (2000-2006) and Alderman Cave, Roswell, NM (2007). Supplements were fed at a rate of 908 g/d in 5 of the 7 years, while cows were fed 1135 g/d in 2003 and 2004. However, the amount of CP supplied was similar each year, regardless of differences in supplementation rate. Composition of the supplements changed slightly over the years; however, supplements consisted mostly of cottonseed meal or wheat middlings and either fish meal and/or hydrolyzed feather meal as the bypass protein source. Supplements were designed to increase in glucogenic potential (**GP**) and provided: 1) 327 g CP, 109-118 g UIP, 44-47 g GP (**CON**); 2) 327-341 g CP, 142-157 g UIP, 57-70 g GP (**BP**); 3) 327 g CP, 151-173 g UIP + 40 – 100 g of propionate salt (NutroCAL^{TM}, Kemin Industries, Inc.), 93-141 g GP (**P**). Supplement P was the only supplement to be fed all 7 years; whereas, CON and BP were only fed 5 and 4 years out of 7, respectively. Glucogenic potential of the supplements was calculated by the equation of Preston and Leng (1987), where 40% of the UIP is considered glucogenic (Overton et al., 1999). NutroCAL^{TM} contains 80% propionate, which has been shown to be 95% glucogenic (Steinhour and Bauman, 1988). All supplements were fortified with macro- and microminerals and vitamin A. Cows and calves had year-long access to a loose salt-mineral mix formulated to complement available forages.

Sampling and Measurements. Blood samples were collected once weekly (2000; Friday) or twice weekly (2001 – 2007) on supplementation days (Monday and Friday) via coccygeal venipuncture beginning 35 to 55 d postpartum for progesterone to estimate days to return of estrus (2 or more consecutive progesterone concentrations $\geq$ 1.0 ng/mL). Blood was collected immediately after cows received supplement. Samples were analyzed for progesterone by solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) as described by Schneider and Hallford (1996). Serum samples were also analyzed for insulin, glucose, non-esterified fatty acid (**NEFA**), serum urea nitrogen (**SUN**) to evaluate nutrient status of each cow. Serum samples were composited by cow within 3 productive peridods: 1) pre-breeding; 2) breeding-end of supplementation; 3) end supplementation-end of breeding. Samples were analyzed using commercial kits for NEFA (Wako Chemicals, Richmond, VA), SUN (Thermo Electron Corp., Waltham, MA), and glucose (enzymatic endpoint, Thermo Electron Corp., Waltham, MA). Insulin was analyzed by solid-phase RIA (DCP kit, Diagnostic Products Corp., Los Angeles, CA). Inter- and intra-assay CV were less than 10% for all serum metabolites.

A subsample of cows (n = 24 in 2000; n = 36 in 2001; n = 29 in 2003; n = 20 in 2004; n = 0 in 2005; n = 29 in 2006; and n = 24 in 2007) were milked with a portable milking machine at approximately 57 d postpartum on a day following supplementation using a modified weigh-suckle-weigh technique described by Appeddu et al. (1997). Milk subsamples were collected into preservative-coated vials for analysis of milk components by an independent laboratory (Pioneer Dairy Laboratories, DHIA, Artesia, NM). Milk weights were calculated for a 24 h milk production.

After calving, cows were weighed once every two weeks (2000 and 2001) or weekly (2003-2007) until the termination of the breeding season, and at weaning. Days to BW nadir was calculated from the lowest BW after calving. Body condition scores (BCS; 1 = emaciated, 9 = obese) were assigned to each cow by visual observation and palpation at initiation of the study, at branding, and at weaning by a trained technician. Calves were weighed at branding and weaning in each year. Branding weights and weaning weights were adjusted for a 55-d branding and 205-d weaning weight with no adjustments for sex of calf or age of dam. Weaning weight for the year following supplementation was not adjusted to show differences in weight caused by variation in calving date and/or conception date.

Economic Analysis. A hypothetical model was developed to compare three 100-cow herds in a two year partial budget of the 3 postpartum supplements using the results from 2000 to 2007. All calves were valued at time of weaning based on a base price in the New Mexico Weekly Weighted Average Feeder Cattle Report (USDA CB LS 795) with no value difference between steers and heifers. In the model, second-year weaning weights were adjusted for calving date by results from days to first estrus and using the weaning weight and days to first estrus for CON as the base. Therefore, weaning weights were adjusted by 0.91 kg for each day decreased in postpartum interval. Calf crop was determined by using the average calf loss according to the SW Cow-Calf Standardized Performance Analysis (SPA) data.

Statistical Analysis. Years were characterized as being either above (**AA**) or below (**BA**) an 18-yr average rainfall for CRLRC. Consequently, year served as the experimental unit for rainfall. However, response to rainfall was not significant and the results were not included. Within each year, there were either two or three supplemental groups. A mixed model accounted for correlations within year and supplement group within year and allowed for appropriate comparison of supplements even though some supplements did not appear in every year. SAS PROC MIXED (ver 9.1.3) was used to analyze the mixed model with cow as the experimental unit and with the fixed effects of supplement, rain, supplement × rain. Year with rain and year within year × supplement were used as the random effects. The Kenward-Roger

degrees of freedom method was used to adjust standard errors and calculate denominator degrees of freedom. Two preplanned contrast statements were used to test for linear and quadratic effects of increasing amounts of glucogenic precursors. The GENMOD procedure of SAS was used to analyze pregnancy rates. Significance was determined at $P \leq 0.10$.

RESULTS AND DISCUSSION

Cows fed P returned to estrus earlier over the 7 years than cows fed any other supplement. A linear decrease ($P = 0.02$) in days to first estrus was found with increasing amount of GP (Table 1). This earlier return to estrus increases the probability that conception will occur earlier in the breeding season (Randel, 1990), which can result in older and heavier calves the following year at weaning. Along with calving earlier the next year, cows will have an opportunity to remain in the herd by becoming reproductively competent sooner and cycling before the initiation of the breeding season. Pregnancy rates increased ($P = 0.01$; 84, 88, and 95% for CON, BP, and P, respectively) with increasing supply of GP in the supplements.

Cow BW and BCS were similar across all treatments at all measurement times ($P \geq 0.16$; Table 1). Treatments did not affect body weight realimentation and therefore, any improvement in reproduction was not associated with a difference in BW gain. Cows reached BW nadir at similar ($P = 0.79$) days postpartum in all supplement groups. However, a linear decrease ($P = 0.07$) in days from BW nadir to first estrus was found with increasing consumption of glucogenic precursors. Once cows reached a positive energy balance, cows fed P required less days to return to estrus.

Serum concentration of glucose increased ($P = 0.02$) linearly with increasing GP in the diet and also decreased ($P < 0.01$) with advancing physiological periods coinciding with improved forage quality. A supplement × physiological period interaction ($P < 0.01$; Table 2) occurred for serum insulin, NEFA, and SUN. Serum insulin concentrations were higher for cows fed BP and P than cows fed CON before breeding season and early in the breeding season. The increase in serum glucose and insulin may have had a positive effect on the restoration of LH pulse frequency as seen by Chagas (2003), who reported decreased days to first estrus and increased pregnancy rates in grazing dairy cows with supplementation of glucogenic precursors. Serum NEFA were higher in the first 2 physiological periods which follows the same trend of cow's weight loss after calving. Cows fed BP tended to have higher SUN concentrations until the last physiological period in which BP had the lowest SUN concentrations compared to cows fed CON and P.

Twenty-four hour milk production exhibited a quadratic response ($P = 0.04$; Table 1) to increasing consumption of GP. A quadratic response was also observed for milk protein, lactose, solids non-fat, and butterfat ($P \leq 0.05$). The additional GP in the BP supplement seemed to be used to promote higher milk production. Supplement P contained the same amount of UIP as the BP supplement, but cows fed P tended to shift nutrients away from milk production and toward reproduction. The increase in 24-h milk production in the BP group agrees with other finding that UIP-supplemented cows produce more milk (Appeddu et al., 1997). The contradicting results between BP and P supplements may be explained by the cows fed BP in our study may not have received enough glucogenic precursors to overcome the effects of insulin insensitivity and passively partitioned nutrients towards lactation. Milk production differences did not alter calf BW at branding ($P = 0.82$). Calf weaning weight followed the same quadratic milk production response ($P = 0.07$) with calves from BP cows having the heaviest weaning weight.

A ranch's productivity can be described as pounds of calf weaned per exposed female (Ramsey et al., 2005). Increasing total kilograms of calf weaned per cow exposed to a bull is a crucial criterion for beef cattle producers and is primarily controlled by reproductive efficiency and calf death loss. Feeding young lactating beef cows supplement P decreased days to first estrus and increased pregnancy rates providing the opportunity to wean older/heavier and more calves the next year. Total kg of calf weaned for the supplemental year and the subsequent year was greater ($P = 0.07$; Table 1) for P fed cows than the other supplements.

Economic Analysis. An evaluation of potential revenue from three 100-cow herds was conducted with a 2-yr partial budget of the 3 postpartum supplements using the results from 2000 to 2007 (Table 3). Feed costs for the supplemental period were \$22.26, \$26.95, and \$33.18/cow for CON, BP, and P, respectively. In yr 1, total revenue was \$21.35 and \$6.44 per cow higher for BP and P, respectively compared to CON. This higher revenue in yr 1 was due to an increase in calf weaning weight in the BP and P cow herds. Pregnancy rates across the 7 yr averaged for the supplement year (yr 1) were 84%, 88%, and 95% for CON, BP, and P, respectively. Consequently, cows fed P in yr 1 had an increase in total revenue of 15.3% compared to CON-fed cows and 7.2% compared to BP-fed cows in yr 2. This increase in revenue is the sum of an increase in pregnancy rates and to a lesser extent a decrease in days to first estrus which offset the higher postpartum feed costs for the year.

IMPLICATIONS

Increasing glucogenic precursors in range supplements decreased days to first estrus and improved pregnancy rates in 2- and 3-yr-old cows by apparently repartitioning nutrients away from lactation. Additionally, the increase in pregnancy rates for cows fed propionate salts offset the higher cost of the supplement by increasing calf crop the following year which increased ranch revenue compared to the other supplements fed in this study.

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Table 1. Effect of postpartum supplementation on reproductive measurements, cow body weight and change, body condition score, milk production, and calf weight change for 2- and 3-yr-old cows grazing native range in 2000-2007.

	Supplement					Contrast	
Measurement	CON	BP	P	SEM	*P*-value	Linear	Quadratic
Reproductive Measurements							
Days to First Estrus	88	87	82	9	0.07	0.02	0.85
Days to Nadir	52	54	52	8	0.87	0.99	0.59
Nadir to Estrus	36	33	29	12	0.19	0.07	0.85
Pregnancy, %	84	88	95	--	0.01	--	--
Cow Wt, kg							
Initial	431	435	436	12	0.60	0.40	0.71
Begin of Breeding	376	374	379	10	0.66	0.39	0.71
Nadir	345	345	348	11	0.80	0.51	0.88
End of Breeding	407	404	410	10	0.54	0.32	0.57
Weaning	444	439	447	12	0.49	0.31	0.48
Body Condition Score							
Initial	4.6	4.7	4.7	0.3	0.40	0.34	0.38
Brand	3.9	4.1	4.0	0.1	0.16	0.23	0.16
Weaning	4.6	4.7	4.7	0.1	0.42	0.31	0.44
Milk, g/d							
24 h Production	6,272	7,136	6,461	441	0.12	0.93	0.04
Protein	164	189	174	13	0.15	0.70	0.05
Fat	215	273	226	21	0.02	0.82	0.01
Lactose	313	358	321	23	0.09	0.82	0.03
SNF	530	611	553	40	0.09	0.93	0.03
Calf Weights, kg							
Branding Wt	66	67	66	4	0.82	0.82	0.56
Weaning Wt	209	218	215	12	0.07	0.15	0.10
kg weaned	207	188	215	25	0.28	0.17	0.32
2 yr total kg weaned	418	410	435	40	0.18	0.07	0.58

Table 2. Supplement × physiological period interactions for serum insulin, non-esterified fatty acid (NEFA), and serum urea nitrogen (SUN) of 2- and 3-yr-old postpartum cows grazing native range and fed supplements increasing in glucogenic precursors in 2000-2007.

		Supplement			
Measurement	Period	CON	BP	P	SEM
Serum Metabolite					
Insulin, ng/mL	Prebreeding	1.34^{x}	1.39^{x}	1.61^{x}	0.54
	Breeding - End Supplementation	1.40^{x}	1.65^{y}	1.73^{y}	0.54
	End Supplementation - End Breeding	1.28^{x}	1.18^{z}	1.38^{z}	0.54
NEFA, µmol/L	Prebreeding	362^{ax}	437^{bx}	408^{bx}	57
	Breeding - End Supplementation	374^{ax}	318^{by}	396^{ax}	57
	End Supplementation - End Breeding	253^{ay}	278^{ay}	255^{ay}	57
SUN, mg/100 mL	Prebreeding	9.76^{ax}	11.07^{bx}	9.72^{ax}	1.35
	Breeding - End Supplementation	8.97^{ax}	10.98^{bx}	9.14^{ax}	1.35
	End Supplementation - End Breeding	12.67^{ay}	10.51^{bx}	12.13^{ay}	1.35

^{a,b} For each interaction, means in rows with different superscripts differ ($P < 0.10$).
^{x,y,z} For each interaction, means in columns with different superscripts differ ($P < 0.10$)

Table 3. A model results comparing cost and revenue for 3 postpartum supplementation strategies for three 100-cow herds for 2 consecutive years. Data from 2- and 3-yr-old cow postpartum supplementation studies (2000-2007) at NMSU's Corona Range and Livestock Research Center were used to construct the 2-year partial budget.

Year 1	**CON**	**BP**	**P**
No. of Cows	100	100	100
Cost of supplement, $/ton	318	364	474
Days of Postpartum Supplementation	70	70	70
Cost of supplement/day	0.318	0.385	0.474
Postpartum supplement cost/cow	22.26	26.95	33.18
Weaning Weight, kg	209	218	215
Price of calves, $/45.4 kg	124	124	124
Weaned calf value, $	569.16	595.2	586.52
Minus Feed Cost, $	546.90	568.25	553.34
Total Revenue/100 hd, $	54,690	56,825	55,334
Difference, $	--	**2,135**	**644**
Pregnancy rates, %	84	88	95
Calving death loss based on exposed females, %	2.8	2.8	2.8
Calf Crop, %	81.2	85.2	92.3
Year 2			
No. of Cows	81	85	92
Estimated Calving Interval, d	365	364	359
Cost of supplement, $/ton	318	385	474
Days of Postpartum Supplementation	70	70	70
Cost of supplement/day	0.318	0.385	0.474
Postpartum supplement cost/cow	22.26	26.95	33.18
Adjusted Weaning Weight for calving date, kg	209	219	220
Price of calves, $/45.4 kg	124	124	124
Weaned calf value, $	569.16	597.68	601.4
Minus Feed Cost, $	546.90	570.73	568.22
Total Revenue/cow herd, $	44,298.90	48,512.05	52,276.24
Difference, $	--	**4,213.15**	**7,977.34**

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IMPACT OF BODY CONDITION SCORE ON REPRODUCTIVE PERFORMANCE IN YOUNG POSTPARTUM RANGE COWS GRAZING NATIVE RANGE

J. T. Mulliniks[1], S. H. Cox[1], M. E. Kemp[1], R. L. Endecott[2], R. C. Waterman[3], D. M. VanLeeuwen[1], and M. K. Petersen[3]

[1]New Mexico State University, Las Cruces, NM
[2]Montana State University, Miles City, MT
[3]USDA-ARS, Fort Keogh Livestock and Range Research Laboratory

ABSTRACT: Body condition score (**BCS**) is used as a management tool to predict reproduction of young beef cows. The objective was to determine the effects of BCS at calving on pregnancy rates, days to first estrus (**DTFE**), nutrient status (assessed by blood metabolites), and calf BW change in 315, 2- and 3-yr-old cows grazing native range over 5 yr at the Corona Range and Livestock Research Center, NM. Palpable BCS (1 to 9) were determined by experienced technicians prior to calving. Cows were assigned to 1 of 4 BCS groups: Thin (**T**; BCS = 3.5 to 4.25), Thin-Moderate (**TM**; BCS = 4.5), Moderate-Fat (**MF**; BCS = 4.75 to 5.25), or Fat (**F**; BCS = 5.5 to 7.0). Postpartum supplementation was terminated each year when cows reached BW nadir. Cows were weighed weekly and serum was collected 2×/wk for progesterone analysis to estimate DTFE. Year effects were also evaluated, with years identified as either above (**AA**) or below (**BA**) average rainfall. Data were analyzed as a 4 × 2 factorial. A calving BCS × rainfall interaction occurred for DTFE (P = 0.01). In AA years, all BCS groups achieved DTFE within 86 d postpartum with F cows cycling the earliest at d 68 postpartum. In contrast, during BA years, cows with a greater BCS (MF and F) took up to 61 d longer postpartum to reach DTFE compared to cows in a thinner BCS (T and TM). Pregnancy rates did not differ between BCS (P = 0.45; 90, 95, 90, 90 for T, TM, MF, and F, respectively). Calf weights at birth (P = 0.19), branding (P = 0.27), weaning (205-d weight; P = 0.51) were not affected by cow BCS at calving**.** Results suggest that BCS interacts with rainfall and may not be a consistent indicator of reproductive performance in young beef cows.

Key Words: beef cattle, body condition score, reproduction

INTRODUCTION

Postpartum interval can have a major economic impact on cow/calf producers in terms of cow productivity. Mature cows tend to be more resilient and have a shorter postpartum interval than young cows (Wiltbank, 1970). A prolonged postpartum interval is more frequent in young cows because of the additional demands for continued growth combined with the stress of lactation. Thus, prepartum body energy reserves can be very important for the resumption of luteal activity and be a useful indicator of nutritional status and reproductive efficiency (i.e., days to first estrus and pregnancy rates) in young cows (Spitzer et al., 1995). Cows with greater body condition before calving respond with improved reproductive performance (Selk et al., 1988; DeRouen et al., 1994; Looper et al., 2003). Guidelines suggest that young cows need to be in a BCS $\geq$ 6 for optimal reproductive performance (DeRouen et al., 1994). Therefore, one objective was to determine the effects of BCS at calving on pregnancy rates, days to first estrus, nutrient status (assessed by blood metabolites), and calf BW change in 315, 2- and 3-yr-old cows grazing native range. Another objective was to determine the interaction of rainfall on reproductive performance, nutrient status, and calf BW in young beef cows.

MATERIALS AND METHODS

Over 5 yr, 315 crossbred, spring-calving 2- and 3-yr-old cows were used to compare the influence of BCS on reproductive performance at the Corona Range and Livestock Research Center (**CRLRC**), Corona, NM. Average elevation at CRLRC is 2,000 m with an average annual precipitation of 380 mm, approximately 70% of which occurs from May to October. Predominant forages in the experimental pastures were blue grama (*Bouteloua gracilis*) and wolftail (*Lycurus phleoides*), as well as other less dominant grasses and forbs (Knox, 1998). The annual standing forage was at least 355 kg/ha in each year and availability was never limiting in all 5 yr (A. Cibils, personal communication).

All animal handling and experimental procedures were in accordance with guidelines set by the New Mexico State University Institutional Animal Care and Use Committee. Management before calving was similar in all 5 yr and between age groups. Prior to calving cows were assigned to 1 of 4 BCS groups: Thin (**T**; BCS = 3.5 to 4.25; palpatable backbone), Thin-Moderate (**TM**; BCS = 4.5; palpatable ribs), Moderate-Fat (**MF**; BCS = 4.75 to 5.25; rib and backbone not palpatable), or Fat (**F**; BCS = 5.5 to 7.0; palpatable fat covering over tailhead). Body condition scores (BCS; 1 = emaciated, 9 = obese) were assigned to each cow by visual observation and palpation at initiation of the study by a trained technician. Cow/calf pairs were moved to a common pasture within 10 d of calving and postpartum supplementation was initiated. Postpartum supplementation provided 36% CP that was fed at a rate of 908 g/cow/d in 4 out of 5 yr or 30% CP fed at 1,135 g/cow/d in 1 yr (cottonseed meal/wheat middlings-based supplement). Total days of supplementation were strategically determined by monitoring average cow BW change within each year. Cows also had ad libitum access

to water and a loose macro- and micro-mineral mix year long. Breeding season started mid-May and ended in less than 60 d. Cows were weighed 1×/wk after calving until the end of breeding and again at weaning. Days to BW nadir were calculated from the lowest BW after calving. Calves were weighed at birth, branding, and weaning in each year. Calf birth weights were recorded in the field with a portable scale within 3 d of birth. Branding weights and weaning weights were adjusted for a 55-d branding and 205-d weaning weight with no adjustments for sex of calf or age of dam.

Serum samples were collected once weekly (1 yr; Friday) or twice weekly (4 yr) on supplementation days (Monday and Friday) via coccygeal venipuncture beginning 35 to 55 d postpartum for progesterone analysis to estimate days to first estrus (2 or more consecutive progesterone concentrations $\geq$ 1.0 ng/mL). Blood was collected immediately after cows received supplement. Samples were analyzed for progesterone by solid-phase radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) as described by Schneider and Hallford (1996). Serum samples were also analyzed for insulin, glucose, non-esterified fatty acid (**NEFA**), and serum urea nitrogen (**SUN**) to evaluate nutrient status of each cow. Samples were analyzed using commercial kits for NEFA (Wako Chemicals, Richmond, VA), SUN (Thermo Electron Corp., Waltham, MA), and glucose (enzymatic endpoint, Thermo Electron Corp., Waltham, MA). Insulin was analyzed by solid-phase RIA (Coat-A-Count; Siemens Medical Solutions Diagnostic, Los Angeles, CA). Inter- and intra-assay CV were less than 10% for all serum metabolites.

Statistical Analysis. Years were characterized as being either above (**AA**) or below (**BA**) an 18-yr average rainfall for CRLRC. Consequently, year served as the experimental unit for rainfall. Within each year, there were either 3 or 4 BCS groups. A mixed model ANOVA accounted for correlations within year and BCS group within year and allowed for appropriate comparison of BCS groups even though some BCS groups did not appear in every year. Data was analyzed as a 4 × 2 factorial. The SAS PROC MIXED (ver 9.1.3) was used to analyze the mixed model with cow as the experimental unit and with the fixed effects of BCS, rain, and BCS × rain. Year within rain and year within rain × BCS were used as the random effects. The Kenward-Roger degrees of freedom method was used to adjust standard errors and calculate denominator degrees of freedom. Two preplanned contrast statements were used to test for linear and quadratic effects of increasing BCS. The GENMOD procedure of SAS was used to analyze pregnancy rates. Significance was determined at $P \leq 0.10$.

RESULTS

A calving BCS × rainfall interaction occurred for days to first estrus (**DTFE**; $P = 0.01$). In AA years, all BCS groups achieved DTFE within 86 d postpartum with F cows cycling the earliest at d 68 postpartum. In contrast, during BA years, cows with a greater BCS (MF and F) took up to 61 days longer postpartum to reach DTFE compared to cows in a thinner BCS (T and TM). Cows in a thinner BCS at parturition tended to return to estrus earlier more consistently than cows in a greater BCS. Body condition score at parturition had no effect on pregnancy rates ($P = 0.45$) or days to BW nadir ($P = 0.74$). These data do not support the conclusion that BCS at parturition is the single most important factor affecting subsequent reproductive performance (Selk et al., 1988; Looper et al., 2003).

Cow BW were similar at the initiation of the study ($P = 0.31$) and at the end of breeding ($P = 0.15$). Body condition score did interact with rainfall for cow BW at the beginning of breeding, BW nadir, and at weaning ($P \leq 0.01$). During AA rainfall years, cow BW of T and TM cows were lighter or similar in BW to MF and F cows. However, in BA years, T and TM cows were heavier than MF and F cows, suggesting that MF and F cows tend to lose more BW after calving than T and TM cows. Cow BW change was not influenced by calving BCS score ($P \geq 0.25$). However, BW change interval from initial BW to beginning of breeding and initial BW to BW nadir tended to exhibit a quadratic ($P = 0.11$; 0.12; respectively) response to calving BCS. Cows in a greater BCS tended to lose more BW than cows in a thinner BCS. Calf weights at birth ($P = 0.19$), branding ($P = 0.27$), and weaning (205-d weight; $P = 0.51$) were not affected by cow BCS at calving, which is consistent with previous results with primiparous beef cows (DeRouen et al., 1994).

Serum glucose concentration was not influenced by calving BCS ($P = 0.90$), which is expected due to the tight regulation of blood glucose (Kaneko, 1997). In contrast, previous research has found an increase in BCS linearly increases concentration of glucose (Vizcarra et al., 1998). A calving BCS × rainfall interaction ($P \leq 0.02$) occurred for serum insulin, NEFA, and SUN. In AA years, insulin was similar for all BCS groups. However, during BA years, serum insulin concentrations were greater for MF and F cows than T and TM cows. In contrast to serum insulin concentrations, NEFA and SUN concentrations were greater in AA rainfall years than in BA years.

Rainfall did not influence pregnancy rates ($P = 0.21$) or days to BW nadir ($P = 0.57$). Cows regained a positive energy balance at similar times which might have influenced the pregnancy rates. Serum glucose concentrations were decreased in lower rainfall years ($P = 0.06$; 55.5 and 50.7 ± 1.6 mg/100 mL for AA and BA, respectively). Cow and calf BW were similar between AA and BA years ($P \geq 0.13$). In contrast, cows in AA years lost more weight from the initiation of the study until the beginning of breeding ($P < 0.01$).

IMPLICATIONS

In this study, body condition score at parturition was not a dominant factor influencing reproductive performance. However, BCS tended to interact with annual rainfall. In years with above average rainfall, all cows tended to perform similarly regardless of BCS. On the other hand, in below-average rainfall years, cows in lower BCS tended to be more resilient to the increase in environmental stress which allowed for a decrease in days from parturition to first estrus. With the variability in annual rainfall pattern of arid climates in the southwestern United States, managing cows to maintain BCS 4.0 to 4.5 at

calving may be more practical to producers on the basis of being more reproductively efficient and more resistant to environmental changes than cows with a greater body condition.

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Table 1. Calving body condition score × rainfall interactions for days to first estrus, serum metabolites, and cow body weight of young cows grazing native range.

		Calving BCS				
Measurement	Rainfall	Thin[a]	Thin-Moderate[b]	Moderate-Fat[c]	Fat[d]	SEM
Days to first estrus	Above Average	86^{ex}	75^{ex}	77^{ex}	68^{ex}	8
	Below Average	73^{ex}	57^{ex}	105^{fy}	119^{fy}	14
Serum Metabolite						
Insulin, ng/mL	Above Average	0.43^{ex}	0.42^{ex}	0.42^{ex}	0.46^{ex}	0.21
	Below Average	1.51^{ey}	1.53^{ey}	2.6^{fy}	2.78^{fy}	0.19
NEFA, µmol/L	Above Average	455^{ex}	478^{ex}	472^{ex}	399^{ex}	63
	Below Average	356^{ey}	399^{ex}	207^{fy}	223^{fy}	55
SUN, mg/100 mL	Above Average	8.5^{ex}	8.4^{ex}	8.5^{ex}	7.8^{ex}	1.5
	Below Average	7.7^{efx}	9.5^{ex}	6.2^{fy}	6.7^{fx}	1.3
Cow BW, kg						
Begin of Breeding	Above Average	371^{ex}	383^{efx}	392^{fx}	384^{efx}	10
	Below Average	407^{ex}	417^{ex}	352^{fy}	378^{efx}	25
Nadir	Above Average	349^{ex}	356^{efx}	364^{fx}	360^{ex}	8
	Below Average	375^{efx}	397^{fy}	320^{gy}	342^{egx}	20
Weaning	Above Average	437^{ex}	444^{ex}	451^{ex}	447^{ex}	9
	Below Average	438^{ex}	466^{ex}	398^{fy}	439^{ex}	24

[a]BCS 3.5 to 4.25
[b]BCS 4.5
[c]BCS 4.75 to 5.25
[d]BCS 5.5 to 7
[e,f,g] For each interaction, means in rows with different superscripts differ ($P < 0.10$).
[x,y] For each interaction, means in columns with different superscripts differ ($P < 0.10$

Table 2. Effects of calving body condition score on reproduction, cow weight and weight change, serum metabolites, and calf weight in young cows grazing native range.

	Calving BCS						Contrast	
Measurement	Thin[a]	Thin-Moderate[b]	Moderate-Fat[c]	Fat[d]	SEM	*P*-value	Linear	Quadratic
Days to BW nadir	59	51	54	53	20	0.74	0.89	0.64
Pregnancy, %	90	95	90	90	--	0.45	--	--
Ratio	26/29	125/132	87/97	51/57	--	--	--	--
Cow BW, kg								
Initial Wt	438	454	434	437	16	0.31	0.74	0.69
End of Breeding	390	411	395	416	15	0.15	0.37	0.97
Cow BW change, kg								
Initial to Begin of Breeding	-48	-54	-62	-55	7	0.25	0.48	0.11
Initial to Nadir Wt	-76	-78	-91	-84	9	0.39	0.46	0.12
Initial to End of Breeding	-47	-42	-39	-22	20	0.27	0.43	0.69
Nadir to End of Breeding	29	36	54	62	16	0.29	0.24	0.43
Nadir to Weaning Wt	76	79	83	93	10	0.50	0.3	0.78
Initial to Weaning Wt	0	1	-8	9	15	0.28	0.75	0.2
Serum Metabolite								
Glucose, mg/100 mL	51.7	53.5	53.4	53.7	3.3	0.9	0.67	0.66
Calf BW, kg								
Birth Wt	33	36	32	31	2	0.19	0.28	0.44
Branding Wt	72	73	64	66	7	0.27	0.35	0.59
Weaning Wt	209	222	195	196	23	0.51	0.59	0.92

[a]BCS 3.5 to 4.25
[b]BCS 4.5
[c]BCS 4.75 to 5.25
[d]BCS 5.5 to 7

Table 3. Effects of annual rainfall (above or below an 18 yr average at the Corona Range and Livestock Research Center) on reproduction, cow body weight and body weight change, serum metabolite and calf body weight in young cows grazing native range.

	Rainfall			
Measurement	Above Average	Below Average	SEM	*P*-value
Days to BW nadir	64	44	23	0.57
Pregnancy, %	93	88	--	0.21
Ratio	221/238	68/77	--	--
Cow BW, kg				
Initial Wt	451	430	11	0.29
End of Breeding	409	397	15	0.59
Cow BW change, kg				
Initial to Begin of Breeding	-69	-41	5	< 0.01
Initial to Nadir Wt	-93	-71	9	0.14
Initial to End of Breeding	-43	-32	23	0.75
Nadir to End of Breeding	51	39	19	0.67
Nadir to Weaning Wt	89	77	11	0.46
Initial to Weaning Wt	-5	5	18	0.70
Serum Metabolite				
Glucose, mg/100 mL	55.5	50.7	1.6	0.06
Calf BW, kg				
Birth Wt	33	33	2	0.98
Branding Wt	62	75	4	0.13
Weaning Wt	196	215	26	0.63

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IMPLICATIONS OF GOING AGAINST THE DOGMA OF FEED THEM TO BREED THEM[1]

A. J. Roberts, E. E. Grings, M. D. MacNeil, R. C. Waterman, L. Alexander and T. W. Geary
USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT

ABSTRACT: Effects of providing differing levels of harvested feed during postweaning development and subsequent winters on reproduction, BW, BCS, and calf BW were evaluated in heifers produced over a 7-yr period from dams fed levels of harvested feed from Dec to March that were expected to be marginal (MARG) or adequate (ADEQ), based on average quality and availability of winter forage. Heifers were either fed to appetite (CON) or restricted fed at 80 % of that consumed by CON on common BW basis (REST) for 140-d period from about 2 mo after weaning to 1 mo before breeding. Heifers were managed together through breeding until Dec when they were separated so CON could be fed adequate harvested feed and REST could be fed marginal levels of harvested feed until 2 to 3 wk before start of calving in March. Cows remained in their treatment through subsequent winters until removed for failure to reproduce or wean a calf. Percent of heifers becoming pregnant and remaining at start of 2nd breeding season was not influenced by dam or heifer treatments ($P > 0.23$; total df = 631). Retention to start of 3rd breeding was less ($P = 0.01$) in REST (58 %) than CON (69 %). Interaction of dam and cow treatments ($P < 0.07$) influenced retention to 4th and 5th breeding. Retention to 4th breeding was less ($P < 0.1$) for REST cows from ADEQ dams (46 %) than the other dam by cow treatment groups (57 to 62 %). Retention to 5th breeding was less for REST cows from ADEQ dams (39 %; $P < 0.01$) than REST cows from MARG dams (66 %); with CON cows from MARG (50%) or ADEQ dams (51 %) being intermediate. Weight and BCS at start of each breeding was 10 kg and 0.10 BCS less ($P < 0.01$) for REST than CON cows. At start of 3rd, 4th and 5th breeding, cows from MARG dams were 15 to 24 kg heavier ($P < 0.01$) than cows from ADEQ dams. Calves from REST cows and MARG granddams were lighter ($P < 0.01$) at birth and weaning by 1.0 and 6.9 kg, respectively, than calves from the other groups (interaction $P < 0.06$). Productivity of cows managed on 2 levels of harvested feed inputs was influenced by the level of harvested feed provided to their dams; greatest feed input did not maximize long term retention.

Key Words: Heifer development, Pregnancy, Retention

Introduction

Feed resources consumed by the cowherd are a major cost associated with beef cattle production. This is especially true for rangeland-based production settings were harvested feed is provided to supplement developing heifers and pregnant cows through periods when quality and quantity of rangeland forage may be limiting. An abundance of research concerning the influences of nutrition on heifer development and cow reproductive performance has resulted in guidelines on body conditions that reflect a nutrient status that will optimize reproductive performance (R. D Randel 1990; Dunn and Moss, 1992; Wettemann et al., 2003). However, a major limitation of the research is a focus on short term effects (single production year) without consideration of long-term implications. There is little doubt that providing cows with sufficient feed can maximize the probability of successful reproduction. However, this may not result in maximum biological and economical efficiency. Providing adequate feed to maximize reproductive rate does not result in differential retention between females with high and low feed requirements that remain in the cow-herd. For example, cows with high feed requirement would more likely be culled for reproductive failure when managed under reduced feed inputs. Likewise, increasing the proportion of cows with reduced feed requirements may provide producers a margin of safety at times when feed resources are scarce or costly. In addition to reducing cost of development, rearing animals under caloric restriction may prolong lifespan, as has been shown in other species (reviewed in Speakman and Hambly, 2007), and as has been suggested for cattle (Hughes et al., 1978).

The present research is a portion of a long-term project to evaluate the influence of 2 levels of nutritional input during heifer development and winter supplementation on lifetime productivity. Objectives of this research were to evaluate the impact of the 2 levels of nutritional input on reproductive performance, BW, BCS, and BW of calves at birth and weaning in females produced over the first 7-yr of the study.

Materials and Methods

All research protocols used in this study were approved by our institutional Animal Care and Use Committee. Cows used in this study were a stable composite population (**CGC**; ½ Red Angus, ¼ Charolais, ¼ Tarentaise). Females studied represent a randomly selected population produced over a 7-yr period (2002 through 2008) by mating CGC dams and sires (n = 62) with consideration given to minimize inbreeding, but without emphasis on production traits. Beginning in the fall of 2001, all cows in this herd were randomly assigned to be fed levels of harvested feed from Dec to March of each year that were expected to be marginal (MARG) or adequate (ADEQ),

[1]USDA-ARS is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA or the authors and does not imply its approval to the exclusion of other products that may be also suitable.

based on average quality and availability of winter forage. Each group of cows was managed on separate pastures during the winter to allow differential feeding. For the majority of the winters in this study, pasture forage was readily available for grazing and the only additional harvested feed provided was alfalfa cake or hay, depending on year, as a supplemental source of protein. This supplement was fed either daily or every other day to achieve an average of about 1.8 kg/d for each ADEQ cow and an average of about 1 kg/d for each MARG cow. During days when access to pasture forage was limited due to snow covering, cows were fed at a rate equivalent to 10.9 or 9.1 kg alfalfa hay/d for each cow in the ADEQ or MARG treatments, respectively.

Each year at weaning, heifer calves were stratified into groups based on weaning weight and were randomly assigned to 1 of 4 (Yr 1) or 1 of 22 to 24 pens (subsequent years). In Yr 1, heifers were group fed with 26 or 27 heifers/pen. Heifers in Yr 2 through 7 were individually fed in pens that contained 6 individual feed bunks equipped with electronic Calan gates (American Calan, Northwood, NH). Heifers were allowed a minimum of 1 mo for adaptation to experimental pens (all years) and to become trained to the head gates (Yr 2 to 7). During this time, heifers were allowed ad libitum access to the test diet fed (described below) once daily. In Yr 1, pens were randomly assigned to receive either control (n=2) or restricted (n=2) level of feeding. In Yr 2 to 7, heifers were randomly assigned within pens to either a control or restricted level of feeding for a 140-d trial. Feed restriction was initiated when heifers were approximately 8 mo of age and 227 $\pm$ 21 kg BW. Control heifers (CON) were fed to appetite and restricted heifers (REST) were fed at 80 % of that consumed by controls adjusted to a common BW basis, as determined at 4-wk intervals using the following formula: [0.80 x (mean BW of restricted/mean BW control) x mean daily feed intake (as fed basis) of controls over the 28-d period]. Total numbers of heifers in each treatment by dam treatment classification for Yr 1 through 6 are shown in Table 1. For calves born in Yr 7, (2008), data are currently limited to BW at birth and weaning.

Composition of the diet fed during the postweaning period is shown in Table 2. Weight of feed offered was recorded daily. Orts were removed from the feed bunk and weight recorded as necessary to ensure that fresh feed was provided for each heifer on a daily basis.

At the end of the 140-d trial, heifers were combined and managed together through breeding and subsequent grazing season. At approximately 14 mo of age (30 to 40 d after end of restriction), heifers from Yr 1 to 4 were weighed and subjected to an estrous synchronization protocol to facilitate breeding by AI followed by natural mating for the remaining duration of a 48- to 53-d breeding season . In Yr 5 and 6, heifers were subjected to a 62-d breeding season with natural mating only. In late Nov to early Dec of each year, pregnant heifers were separated back into their treatment groups to allow for provision of harvested feed at the same levels as described above for the cows; where CON heifers were fed what was expected to be adequate level harvested feed and REST heifers were fed a marginal level of harvested feed. These winter feeding treatments continued until 2 to 3 wk before start of calving in March, when heifers were recombined and managed together. Females remained in their treatment through subsequent winters until removed for failure to reproduce or wean a calf. Percent of heifers becoming pregnant and remaining in the herd at start of each breeding season was recorded. Birth weight and weaning weight were measured on calves produced by females on the different treatments.

Data were analyzed with the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Influence of treatment and dam treatment on reproduction, BW, BCS, and BW of calf at birth and weaning were analyzed using a model that included year of birth, treatment, dam treatment and the interaction of these fixed effects. Least square means and SE are presented, unless specified otherwise.

Results and Discussion

Feed intake and growth characteristics of heifers developed on the two levels of feeding have been reported previously for Yr 2, 3 and 4 (Roberts et al., 2007). As was reported for these 3 years, restricted fed heifers consumed 27% less feed over the 140-d trial resulting in a 26 kg lighter ($P <$ 0.001) BW at the end of the trial. Differences in BW of restricted and control fed heifers persisted ($P < 0.01$) throughout the pre-breeding period (316 vs. 338 $\pm$ 2 kg at approximately 13.5 mo of age) and subsequent grazing season (404 vs. 414 $\pm$ 2 kg at about 19.5 mo of age). Although ADG was reduced during feed restriction, ADG from end of the 140-d trial to 19.5 mo of age was greater ($P < 0.01$) in restricted heifers than control heifers (0.49 vs. 0.42 $\pm$ 0.005 kg/d), indicative of a compensatory response. Weight (Figure 1) and BCS at start of breeding at 2 to 5 yr of age, was 10 kg and 0.10 BCS less ($P <$ 0.01) for REST than CON cows. Thus, the REST protocol used in this study resulted in lighter BW of cows throughout 5 yr of age. This appears to be due, at least in part, to lower BCS. At 3, 4 and 5 yr of age, cows from MARG dams were 15 to 24 kg heavier ($P < 0.01$) than cows from ADEQ dams (Figure 1). These data indicate that BW of a cow may be influenced by level of winter supplemental feed provided to its dam.

Retention of females out to the 5th year of breeding is depicted in Figure 2. Percent of heifers becoming pregnant in their first breeding season (Yr 1 Figure 2) and proportion remaining in the herd at start of 2nd breeding season (Yr 2 in Figure 2) was not influenced by dam or heifer treatments (P of model > 0.23). Retention to start of 3rd breeding was less ($P =$ 0.01) in REST (58 %) than CON (69 %) cows. Interaction of dam and cow treatments ($P < 0.07$) influenced retention to 4th and 5th breeding. Retention to 4th breeding was less ($P < 0.1$) for REST cows from ADEQ dams (46 %) than the other dam by cow treatment groups (57 to 62 %). Retention to 5th breeding was less for REST cows from ADEQ dams (39 %; $P < 0.01$) than REST cows from MARG dams (66 %); with CON cows from MARG (50%) or ADEQ dams (51 %) being intermediate. While not statistically different until Yr 3, the numeric trend for Yr 1 and 2 is for the REST cows to have fewer retained, which is most obvious in cows from ADEQ dams (solid black vs. solid gray bars in Figure 2). It is expected that these experimental treatments would be most similar to experimental conditions evaluated in previous research, where level of nutrition of dam has not generally been considered, but most likely was managed

for optimal production. In this respect, the comparison of CON cows from ADEQ dams to REST cows from ADEQ dams fits the results expected based on previous research concerning nutritional effects on reproduction. Furthermore, results indicate that the negative effects appear to be cumulative over the 5 breeding seasons. However, a novel observation of the present research is the apparent influence of the dam's level of nutrition on its offspring's response to nutritional treatment. While number of cows that are old enough to have observations for retention out to 4 and 5 breeding seasons may be somewhat limited, the data indicate that managing cows on what was expected to be marginal levels of nutrition, improved the ability of their offspring to sustain reproductive performance when they were managed with marginal levels of harvested feed inputs.

Calves from REST cows and MARG granddams were lighter ($P < 0.01$) at birth and weaning by 1.0 and 6.9 kg, respectively, than calves from the other groups (interaction $P < 0.06$; Table 3). As with retention, these results provide evidence that a cow's response to different levels of nutrition may be altered by the nutritional treatments imposed on it's dam. The basis for the small decrease in BW at birth and weaning for calves from REST cows and MARG granddams remains to be determined. However, it is interesting to speculate that this small decrease in output may be contributing to the increased rates of retention out at 5th breeding. While additional data concerning long term retention are needed, current trends indicate that the small decrease in calf output may be more than compensated by increased longevity.

Implications

Productivity of cows managed on 2 levels of harvested feed inputs was influenced by the level of harvested feed provided to their dams; greatest feed input did not maximize long term retention. Thus, feeding to maximize short term reproductive performance or any other trait may not equate to the greatest production efficiency in the long term. In this respect, greater efficiency is probably achieved by matching the genetics to the environment rather than altering the management (increase feed inputs) to support changes resulting from genetic selection. This research also provides evidence that nutritional influences on replacement heifers may begin in utero, or earlier, and continue throughout life. Maintaining cows under a marginal nutritional environment through the winter and developing their heifers on lower levels of nutrient input may improve efficiency and enhance longevity.

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Table 1. Year of birth (YOB) and number of control and restricted fed heifers from dams that were provided adequate (ADEQ) or marginal (MARG) levels of harvested feed throughout the winter

		Control		Restricted	
Yr	YOB	ADEQ	MARG	ADEQ	MARG
1	2002	21	31	30	23
2	2003	31	32	34	28
3	2004	43	43	44	38
4	2005	39	30	35	31
5	2006	36	37	38	33
6	2007	36	31	34	34
All		206	204	215	187

Table 2. Composition (%, DM basis) of diets fed during the 140-d feeding period for Yr 1 and range of composition for Yr 2 to 7

	Yr 1	Yr 2 to Yr 7
Corn silage	52	67 to 68
Alfalfa	38	17 to 18
Supplement[1]	10	15
DM	47.5	36 to 37
CP	13.3	15 to 18

[1]Containing protein and mineral.

Table 3. Influence of level of nutrition provided to granddam and dam on BW of calves at birth and weaning[1]

Granddam treatment	Dam treatment	BW at birth, kg	BW at wean, kg
ADEQ	CON	35.0	203.6
ADEQ	REST	35.0	202.3
MARG	CON	35.0	201.4
MARG	REST	33.6[2]	196.4[2]

[1]See figure legends for description of nutritional treatments.
[2] Differs ($P < 0.01$) from other groups.

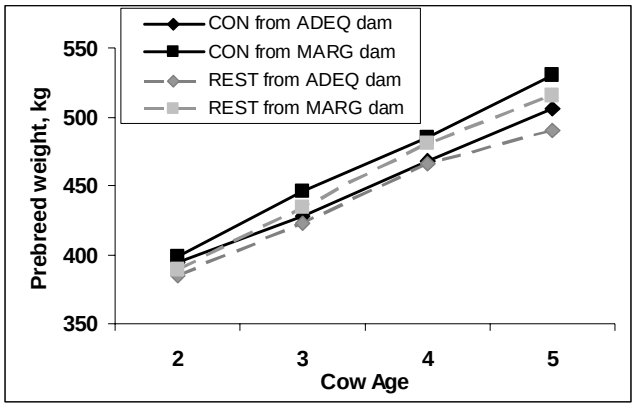

Figure 1. Effect of level of feed inputs provided during postweaning development and annual winter supplementation and by level of winter feed inputs provided to dams on BW of cows prior to breeding at 2 to 5 yr of age classified by. Cows developed with ad lib access to feed and provided adequate winter harvested feed inputs (CON, black lines) were heavier ($P = 0.01$) than cows developed with restricted feed intake and provided marginal levels of harvested feed in the winter (REST; grey lines). At 3, 4 and 5 yr of age, cows from dams provided marginal levels of harvested feed in the winter (MARG, square symbols) were heavier ($P < 0.01$) than cows from dams provided adequate levels of harvested feed during the winter (ADEQ, diamond symbols).

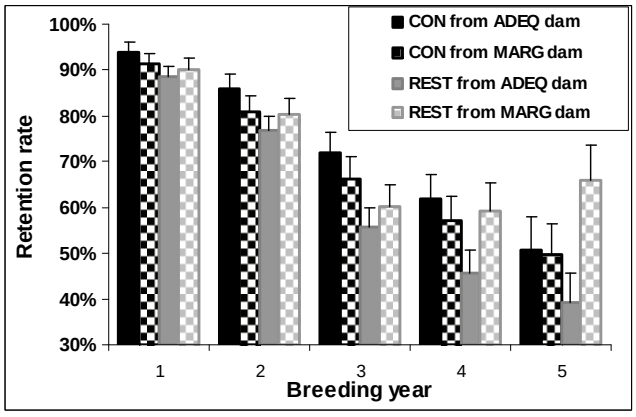

Figure 2. Retention of cows classified by level of feed inputs provided during postweaning development and annual winter harvested feed inputs and by level of winter feed inputs provided to their dams. Heifer calves born to dams that had been fed levels of harvested feed from Dec to March of each year that were expected to be marginal (MARG, depicted by square symbols in bars) or adequate (ADEQ, depicted by solid bars) were randomly assigned to be fed ad libitum (CON, black bars) or restricted (REST; gray bars) access to feed during a 140-d trial after weaning, and then were subsequently fed adequate or marginal of harvested feed from Dec to March of each year. Number of animals represented for each breeding year is dependant on number years elapsed since year of birth, and thus numbers evaluated decline each year (n = 776, 632, 505, 385 and 226 for Yr 1 though 5, respectively) accounting for the disconnect between breeding years. Values shown for Yr 1 are heifer pregnancy rates. Values for Yr 2 through 5 are proportion remaining at beginning of 2nd through 5th breeding season. Retention did not differ among groups in Yr 1 or 2, but was greater in CON (black) than REST (gray) cows at Yr 3 ($P = 0.01$). Treatment by dam treatment interactions were evident for Yr 4 and 5 ($P = 0.07$ and 0.04, respectively). In Yr 4, retention was less for REST cows from ADEQ dams than other groups ($P < 0.1$). In Yr 5, retention of REST cows from ADEQ dams ($P = 0.005$) and CON cows from MARG dams ($P = 0.09$) was less than for REST cows from MARG dams.

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RAM MANAGEMENT PRACTICES: RESULTS OF A 2008 WYOMING SHEEP PRODUCER SURVEY

V.A. Uthlaut*[1], J.P. Hewlett[2], P. Jain[2], B.S. Rashford[2], B.M. Alexander[1]

Departments of Animal Science[1] and Agricultural and Applied Economics[2], University of Wyoming, Laramie

ABSTRACT: Wyoming sheep producers were surveyed to determine the perceived importance of ram sexual behavior in their management practices. The survey was developed to gather general ranch and herd management information. The National Agricultural Statistics Service (**NASS**) provided the sampling frame from producer lists kept by NASS. Wyoming sheep producers (n = 719) received the survey of which 47% responded and was representative across small (< 30 ewes), small to medium (30-49 ewes), medium (50-99 ewes), medium to large (100-299 ewes) and large (> 299 ewes) sized operations. Although producers had a high level of agreement on the importance of breeding performance (mode of 5 "strongly agree"; mean 4.4 ± 1.0) for flock productivity, libido was considered the least important criterion for ram selection by 59% of producers, ranking libido third of the three most important criteria for ram selection. Breed (63%), scrapie genotype (31%) and structural soundness (30%) were considered the most important ram selection criteria. Muscling (44%) was ranked as the second most important criteria for ram selection. Similar to libido, feedlot test performance (56%) and pedigree/genetics (51%) were ranked third. Wool characteristics and breeding soundness exam were of less importance for ram selection. Rams are most commonly culled due to increased age (mode of 5; mean 4.4 ± 1.0), but lack of libido was an important consideration (mode of 5; mean 3.2 ± 1.4). Sheep producers were neutral in their perception of current feedlot performance tests adequately evaluating the breeding competence of rams (mode of 5; mean 3.4 ± 1.2). This survey indicates although producers are aware of differences in ram libido that would affect flock performance, they have not incorporated breeding performance as an important criteria for ram selection. Furthermore, some producers erroneously believe that current feedlot performance tests evaluate breeding competence.
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Key Words: Rams, Libido, Breeding performance, Feedlot test performance.

Introduction

A Wyoming Sheep Producer Survey was developed to gather information about sheep production in Wyoming. General ranch information including herd, ram and predator management practices as well as general demographics were collected. A subsection of the survey concentrated on ram management practices. This section contained five questions designed to elicit information about the importance of ram performance, culling, criterion for selecting rams and flock composition.

The influence of ram mating behavior on conception and lambing rates has been demonstrated in pen (Perkins et al., 1992) and field (Mattner et al., 1971; Stellflug et al., 2006) mating trials. Ram selection based solely on production traits may cost producers economic opportunities as well as slow genetic progress of the flock if those selected rams have a low propensity for sexual performance. Of the 196,000 rams nationwide, approximately 23% of all rams are predicted to be non-breeders (Fitzgerald and Perkins, 1992) resulting in an annual loss of $13.5 million in ram costs alone to U.S. sheep producers. Researchers at the U.S. Sheep Experiment Station suggest identification of both low- and non-performing rams would reduce the number of rams required for breeding flock maintenance by 50% (ASI, 2005).

This survey was conducted to gather sheep production information with an emphasis on ram management practices. Current management practices must be assessed to determine where changes in management are most likely to be adopted to improve production and profitability through increased vigilance of ram sexual behavior.

Materials and Methods

All Wyoming sheep producers were issued a survey by the National Agricultural Statistics Service (NASS). The producer lists kept by NASS are comprehensive and routinely updated. Because their lists are confidential, the Wyoming office of the USDA's NASS was responsible for administration of both mail and phone surveys.

The initial mailing contained a cover letter, survey and return postage-paid envelope. One week later, all potential respondents received a follow-up postcard asking them to return the survey and thanking them if they had already done so. Two to three weeks after the initial mailing a second mailing was sent out containing a cover letter, another copy of the survey and a postage-paid envelope. Three weeks following the initial mailing, non-respondents were re-sampled and the full instrument was delivered using telephone enumerators. A stratified random sample (n = 719) of sheep producers in the state received the survey, of which 47% responded.

Ram management practices were surveyed by asking the level of agreement to 11 statements. A scale of 1 to 5 was used with 1 = "strongly disagree" and 5 = "strongly agree." Another section asked producers to rank

the most important criterions in ram selection. A final section asked producers the average age of their rams at the first breeding season and number of ewes per ram they typically place in the breeding flock.

Results and Discussion

Size of operation was categorized according to the number of bred ewes reported as typically owned and were defined as: small operations (< 30 bred ewes), small to medium operations (30-49 bred ewes), medium operations (50-99 bred ewes), medium to large operations (100-299 bred ewes) and large operations (≥ 300 bred ewes). Of the 338 valid responses, 125 were from small, 41 from small to medium, 56 from medium, 54 from medium to large and 62 from large operations.

Producers were neutral (3.6 ± 1.2; 1 = "strongly disagree" and 5 = "strongly agree") in their perception of the importance of ram performance testing for genetic progress and profitability of their flocks. Furthermore, producers were not clear (3.4 ± 1.2) whether current performance testing evaluates breeding competence, or libido, of rams. Sheep producers did, however, agree that breeding performance (i.e. libido) was important for flock productivity (4.4 ± 1.0) and rams with proven breeding competence (4.2 ± 1.0) were more valuable than those with unknown breeding competence.

Rams were most commonly culled because of age (4.4 ± 1.0). Lack of libido (3.2 ± 1.4) was not a strong reason for culling rams. Interestingly, lack of libido was a more important reason to cull rams for producers from small sized operations (mode = 5) than medium to large (mode = 2) or large sized operations (mode = 3). Culling rams due to lack of structural soundness, presence of epididymitis or lack of body condition (mean 3.2 ± 1.5) did not produce strong agreement.

Breed was the most important ram selection criterion among all operation sizes (65% of respondants ranked it number 1; Table 1). Scrapie genotype was also an important ram selection criterion (31%) as was structural soundness (30%). Muscling (44%) was the most common 2nd ranked criterion for ram selection. Structural soundness, wool characteristics and breeding soundness exams were also commonly ranked 2nd (Table 2). Overall libido was considered the least important criterion with 59% of respondents ranking it 3rd. As with reasons for culling, libido was a more important criterion for ram selection among small sized operations (27% ranked it first) than with medium or medium to large sized operations (0% ranked first). Feedlot test performance was commonly ranked 3rd as was pedigree and "other" criteria.

Rams are 15 ± 7 mo of age during their first breeding season, a characteristic consistent across all operation sizes. As expected, numbers of ewes expected to be bred by each ram varied by operation size (Table 2). Operations with < 30 head of ewes had a smaller ewe to ram ratio which increased with greater ewe numbers.

Implications

This survey indicates that although Wyoming sheep producers are aware of differences in ram libido that would affect flock performance, they have not incorporated breeding performance as an important criteria for ram selection. Furthermore, some producers erroneously believe that current feedlot performance tests evaluate breeding competence.

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Table 1. Ranking percentages for the three most important criterions in selecting rams: all ranches.

Characteristics[a]	Rank 1	Rank 2	Rank 3
Breed	63%	14%	22%
Scrapie Genotype	31%	34%	34%
Muscling	25%	44%	31%
Structural Soundness	30%	41%	29%
Wool Characteristics	27%	38%	35%
BSE Results	28%	31%	41%
Libido	18%	23%	59%
Feedlot Test Performance	22%	22%	56%
Pedigree/Genetics	20%	29%	51%
Other	33%	11%	56%

[a]Producers were provided a list of characteristics and were asked to rank three most important criterions in ram selection. Rank1 is assumed to be the most important trait while rank 3 the least important criterion. Percentages equal 100% across characteristics.

Table 2. Average number of ewes typically planned to be covered by each breeding ram.

	Operation Type				
All Ranches n = 309	< 30 Bred Ewes n = 81	30-49 Bred Ewes n = 36	50-99 Bred Ewes n = 53	100-299 Bred Ewes n = 53	> 299 Bred Ewes n = 59
27 (15)	15 (8)	27 (10)	28 (10)	32 (11)	38 (18)

Means are rounded to the nearest whole numbers.
Standard deviations are in parenthesis (rounded to the nearest whole number).

FACTORS INFLUENCING THE ADOPTION OF BEST MANAGEMENT PRACTICES FOR FEEDLOT AMMONIA EMISSIONS

C.H. Davidson*, J.G. Pritchett, N.M. Embertson, and J.G. Davis
Colorado State University, Fort Collins, CO

ABSTRACT: Ammonia emissions from feedlot operations pose risks to human and ecosystem health. In particular, nitrogen deposition in Colorado's Rocky Mountain National Park has been associated with livestock feeding in the western Corn Belt and Colorado. Feedlot operators can implement a variety of Best Management Practices (BMPs) to reduce ammonia emissions. These BMPs vary in simplicity, managerial time, effort and required financial capital. Although the ammonia-mitigating potential of various BMPs is well-researched, little research examines the barriers that prevent feedlot operations from adopting the BMPs. To learn more about these barriers, a questionnaire was mailed to 1,998 dairy and feedlot producers in June 2007. Survey responses (overall response rate of 7.6% for feedlots and dairies) allow determination of current levels of BMP adoption as well as producer perceptions of the environmental impact and economic feasibility of each BMP. Of the thirteen BMPs surveyed, six of the BMPs had adoption rates greater than 50%, indicating sizeable overall adoption levels. Probit analysis enables estimation of the conditional probability of adoption given a set of attributes. Explanatory variables in the probit analysis include farm characteristics as well as operator perceptions of cost, profitability, ease of adoption, and environmental impact. The results from the probit model varied substantially across BMPs, with the most robust findings for hiring a nutritionist, implementing group feeding, testing soil for nutrients and providing shade in drylot pens. Practices involving high fixed costs were more likely to be adopted by large operations and by managers that perceive a practice as profit-enhancing.

Key words: Ammonia, Best Management Practices, Feedlot

Introduction

Ammonia is produced on livestock operations when urea nitrogen in urine combines with the urease enzyme in feces and rapidly hydrolyzes to form ammonia gas (Muck, 1981). Once in gaseous form, ammonia reacts with other particles in the atmosphere, especially nitric and sulfuric acids produced from vehicles and industrial emissions to form fine particulate matter ($PM_{2.5}$). The small size of these particles enables wind to carry them from rural areas to urban areas, where they build up in the atmosphere contributing to smog and respiratory problems (Marcillac *et al*., 2007).

The U.S. Environmental Protection Agency estimates that approximately 40% of total ammonia emissions in the United States come from livestock (Battye, 1994). If livestock producers were to adopt a combination of Best Management Practices (BMPs), potential ammonia emissions reductions could approach two-thirds (Powell, 2006). Outreach professionals recognize the effectiveness and environmental trades-offs of ammonia BMPs, but have little information on producer adoption and constraints to inform their outreach strategy. Producers are likely constrained by too little investment capital, insufficient cash flows or other barriers. Barriers to adoption likely vary according to the BMP considered.

Previous research on adoption of manure management BMPs primarily focuses on practices targeted at improving water quality. A study of dairy producers (Nunez and McCann, 2008) found that off-farm income, location, perceived profitability and perceived complexity were significant factors in determining adoption of four water quality manure BMPs in Iowa and Missouri. Prior to this research, Rahelizatovo (2002) found that adoption of dairy water quality BMPs was highly influenced by farm and operator characteristics, environmental perceptions as well as producer attitudes. The current research aims to extend this body of research to ammonia BMPs, which, due to different capital, labor and technology requirements likely pose different barriers than water quality BMPs.

Materials and Methods

A survey was designed following Dillman (1991) and mailed to 1,998 feedlots and dairies in Colorado, Iowa, Kansas and Nebraska, resulting in a paltry 7.6% response rate. The survey requested information on producer adoption of thirteen BMPs listed in Table 1. These practices are known to reduce ammonia emissions (Marcillac et al., 2007) though producer knowledge of the practices' benefits may be limited. BMP adoption among the survey respondents is listed in Table 1's second column, and range from heavily adopted (e.g., using feed additives) to those that are seldom adopted (e.g., adding an acidifier to the surface of a dry lot). Each BMP was given an abbreviation that is found in the parenthesis of Table 1.

BMP adoption relies on the attitudes of feedlot managers, manager demographics, feedlot business characteristics, the local institutional environment and other factors. The hypothesized factors influencing adoption in this study are listed in Table 2 along with the manner in which they are obtained (e.g., a dichotomous variable coded as a 1 or 0) and coded for analysis. Summary statistics were tabulated, providing a general profile of the survey sample and are available from the authors upon request.

Table 1. Description and feedlot adoption rate of BMPs.

Best Management Practice	Adoption
Use feed additives (ADD)	96%
Measure crude protein (PROTEIN)	93%
Practice group feeding (GROUP)	88%
Perform yearly soil test (SOIL)[1]	78%
Hire a nutritionist (NUTRITION)	77%
Collect runoff water (RUNOFF)	67%
Remove manure (CLEAN)[2]	60%
Test for nutrients (TEST)[3]	59%
Provide bedding in drylot pens (BED)	52%
Incorporate manure (INCORPORATE)[4]	42%
Provide shade in drylot pens (SHADE)	34%
Apply water to drylot surface (SURFACE)	28%
Apply an acidifier to drylot surface (ACID)	3%

[1] perform yearly soil test for cropland nutrients; [2]remove more than four times per year; [3] test manure, effluent and compost; [4] incorporate within 48 hours of application

Table 2. Description of explanatory variables used to estimate probability of BMP adoption.

Variable	Description
SIZE	Number of cattle raised in the last year
CROP	Acres of cropland
STATE	Dummy; 0= Colorado, 1= other state
IOWA	1= Iowa
KANSAS	1= Kansas
NE	1= Nebraska
REVENUE	Cost efficiency
INVEST	Yearly investment capabilities, $
DIVERSE	Percent revenue from non-feedlot activity
OWN	Percent of cropland owned by respondent
FUTURE	Dummy; 0= invested, 1= divested
RETIRE5	Plans to retire within 5 years
LIVE5	Plans to invest in livestock within 5 years
LIVE15	Plans to invest in livestock within 15 years
EXPER	Number of years managing operation
AGE	Years
EDUC	Years of education starting from 1st grade
PROFIT	Perception of profitability of BMP
COST	Perception of cost of BMP
TECH	Perception of technical need for BMP
WATER	Perception that BMP benefits water
AIR	Perception that BMP benefits air

Of the BMPs listed in Table 1, the respondents' cost perceptions of the BMP, its perceived profitability and the amount of technical expertise that is required are of particular note. These are the variables COST, PROFIT and TECHNICAL respectively in Table 2, and respondents were asked to rate their level of agreement using a Likert type scale (1= strongly disagree … 5 = strongly agree). Raw data are summarized in Table 3. As an example, measurement of crude protein (PROTEIN) is generally found to improve profitability (ranking of 4.4), requires technical assistance (4.0) and is not perceived to be costly (2.6).

The impact that Table 2's variables have on BMP adoption will vary by the practice; after all, BMP's vary in their requirements for capital, cash flow, technical expertise, etc. The general form for the relationship between the BMP and explanatory variables is listed in equation 1.

Table 3. Average operator response for economic perceptions of BMPs (5=strongly agree; 1=strongly disagree).

	Do you think BMP is:		
BMP	Profitable	Technical[1]	Costly
PROTEIN	4.4	4.0	2.6
NUTRITION	4.1	4.2	3.1
ADD	4.3	3.7	3.2
GROUP	4.1	2.1	2.1
SHADE	3.4	2.2	3.4
SURFACE	2.9	2.1	3.4
ACID	2.5	3.0	3.4
CLEAN	3.7	2.1	3.5
BED	3.2	1.9	3.8
RUNOFF	3.0	3.2	3.9
INCORPORATE	3.4	2.2	3.5
TEST	3.8	4.0	3.4
SOIL	4.0	4.2	3.5

[1] requires technical assistance

(1) BMP_i = F (SIZE, CROP, STATE, INVEST, REVENUE, EDUC, DIVERSE, OWN, EXPER, FUTURE, AGE, PROFIT, TECH, COST, AIR, WATER) + e

where subscript i refers to the i^{th} BMP in Table 1, the explanatory variables are described in Table 2, and e is the error term, which is assumed to be distributed logistically.

Discrete choice methods enabled the estimation of factors influencing the probability of adopting a BMP or set of BMPs based on attributes surveyed. Discrete choice modeling is appropriate in this research as the adoption of a BMP is coded as 1 and non-adoption is coded as 0. Probability of adoption is grounded in random utility theory, where a utility-maximizing producer chooses whether to adopt a practice (Greene, 2000; Maddala, 1983).

An initial univariate logit analysis of each of the thirteen BMP adoption equations provides preliminary estimates of the relationship between explanatory variables and adoption rates, as well as identifies candidate variables for the subsequent multivariate analysis. Variables found significant at the 25% level or greater in the univariate analysis are included in the multivariate analysis. Multivariate probit analysis is then used to improve estimate efficiency by allowing for interaction among adoption of practices. The multivariate analysis requires BMPs be grouped, and the BMPs in this study are grouped according to whether they are used in manure application (BMPs include SOIL, TEST and INCORPORATE), in managing the drylot (BED, CLEAN, RUNOFF, SHADE) or managing feed inputs (GROUP, PROTEIN, NUTRITION and ADD). Results for each group are discussed in turn.

Results

Perceptions of profitability (PROFIT) positively impact the adoption of manure application BMPs including performing a yearly soil test (SOIL), testing manure for nutrient values (TEST) and incorporating manure into cropland (INCORPORATE) within 48 hours of application (Table 4). Size of the operation (SIZE) also has positive impacts on adoption meaning that larger operations are more likely to adopt TEST and INCORPORATE, though the relative impact of SIZE is quite small compared to other statistically significant explanatory variables. Respondents who perceive SOIL and TEST to require technical expertise (TECH) are less likely to adopt the practice for that reason. The number of years managing a feedlot (EXPER) contributes to adoption of the BMP TEST. More diversified operations (DIVERSE) are more likely to incorporate manure within 48 hours of application (INCORPORATE).

Table 4. Multivariate probit results for manure application practices.

Variable	Coefficient	St. Error	P-value
1. SOIL			
Constant	1.3589	0.3141	0.0001
SIZE	-1.55E-05	0.0001	0.6662
PROFIT***	0.4021	0.1416	0.0045
TECH***	-0.4018	0.1415	0.0045
KANSAS	-0.7727	0.5096	0.1295
IOWA**	-0.8273	0.3389	0.0146
2. TEST			
Constant	0.1503	0.1569	0.3381
SIZE***	0.0001	1.94E-04	0.0042
PROFIT***	0.5969	0.1864	0.0014
TECH***	-0.5959	0.1864	0.0014
EXPER**	0.0018	0.0014	0.2092
3. INCORPORATE			
Constant	-2.7292	0.5326	0.0001
SIZE**	-5.21E-05	1.62E-05	0.0458
PROFIT***	0.7347	0.1502	0.0001
OWN	-0.0009	0.0006	0.1288
DIVERSE***	0.0013	0.0004	0.0021
Log-likelihood			-206.58
Correlation coefficients[1]			
R(01,02)	0.3026		
R(01,03)	0.0285		
R(02,03)	0.3777		

*** significant at 1% level; **significant at 5% level; *significant at 10% level; [1] indicates correlation between BMP adoption decisions

Managing the drylot includes the BMPs of BED, CLEAN, RUNOFF, and SHADE with results presented in Table 5. Smaller operations are more likely to provide shade (SHADE) and bedding (BED). Cost efficient operations (REVENUE) are more likely to provide shade and remove manure frequently as indicated by the positive sign on SHADE and CLEAN. State location relative to Colorado influences an operator's probability of removing manure frequently- location in Iowa increases the relative likelihood while location in Kansas decreases the relative likelihood. Perceptions of profit (PROFIT) and future plans (RETIRE5, LIVE15, LIVE5) have the most significant impact on a respondents' decision to provide bedding.

Table 5. Multivariate probit results for drylot best management practices.

Variable	Coefficient	St. Error	P-value
1. BED			
Constant	-2.3091	0.4245	1E-07
SIZE*	-1.55E-05	8.46E-06	0.0676
RETIRE5**	0.0009	0.0004	0.0209
LIVE15**	-0.0007	0.0003	0.0326
LIVE5**	0.0007	0.0004	0.0396
PROFIT***	0.8464	0.1427	2.9E-07
DIVERSE	-0.0006	0.0006	0.3365
2. CLEAN			
Constant	0.5893	0.3339	0.0776
SIZE	1.53E-05	2.62E-05	0.5582
PROFIT	0.0011	0.001	0.2732
INVEST	9.07E+03	1.24E-06	0.4641
REVENUE**	0.0006	0.0003	0.0369
EDUC	0.0011	0.0007	0.1258
IOWA***	0.9296	0.2763	0.0008
KANSAS***	-0.9317	0.2698	0.0006
OWN	-0.001	0.0012	0.3947
3. RUNOFF			
Constant	-0.4467	0.2766	0.1063
SIZE***	0.0006	0.0002	0.0033
PROFIT	0.1209	0.1052	0.2502
TECH	-0.121	0.1052	0.2501
INVEST	1.74E-06	4.50E-06	0.6985
REVENUE	-0.0003	0.0003	0.3457
DIVERSE	-0.0008	0.0008	0.2853
OWN	-0.0009	0.0008	0.2194
4. SHADE			
Constant	0.3407	0.2679	0.2036
SIZE***	-0.0001	1.62E-05	0.0013
PROFIT	-0.0023	0.0045	0.6066
COST	0.0019	0.0045	0.6774
CROP	-0.0001	0.0001	0.2845
INVEST	7.53E-06	4.93E-06	0.1262
EXPER	0.001	0.002	0.6201
REVENUE*	0.0005	0.0003	0.0762
DIVERSE***	-0.002	0.0005	0.0001
OWN	0.0015	0.0015	0.3385
Log-likelihood -267.21			
Correlation coefficients[1]			
R(01,02)	0.2128		
R(01,03)	0.3430		
R(02,03)	-0.3000		
R(01,04)	0.2434		
R(02,04)	-0.1551		
R(03,04)	0.0069		

*** significant at 1% level; **significant at 5% level; *significant at 10% level ; [1] indicates correlation between BMP adoption decisions

Of the four feeding BMPs, two models failed to converge (GROUP and PROTEIN), and only the amount of cropland impacts the practice of providing feed additives (ADD) (Table 6). The poor results for ADD could be explained by the lack of variability in adoption

rates, as 96% of respondents use feed additives. Perceptions of cost (COST) and profitability (PROFIT) are found to statistically impact respondents' decision to hire a nutritionist. The perception that the practice is costly decreases the probability of adoption, whereas the perception that the practice is profitable increases the probability of adoption.

Table 6. Multivariate probit results for feeding best management practices.

Variable	Coefficient	St. Error	P-value
1. NUTRITION			
Constant	-1.1792	0.7229	0.1028
SIZE***	0.0008	0.0002	0.0004
PROFIT***	0.5033	0.1375	0.0003
COST*	-0.2819	0.1594	0.077
REVENUE	-0.0003	0.0004	0.3943
AIR	-0.509	0.3633	0.1612
WATER	0.5093	0.3633	0.1609
2. ADD			
Constant	1.6995	1.4406	0.2381
SIZE	0.0003	0.0007	0.7157
CROP*	-0.0001	0.0001	0.0921
PROFIT	-0.0016	0.0804	0.9838
EXPER	-0.0118	0.0343	0.7313
OWN	0.0014	0.0015	0.3421
FUTURE	-0.9359	0.9132	0.3055
REVENUE	0.0002	0.0007	0.8216
STATE	0.7564	1.26	0.5483
Log -likelihood	-60.99		
Correlation coefficient[1]			
R(01,02)	-0.6060		

*** significant at 1% level; **significant at 5% level; *significant at 10% level; [1] indicates correlation between BMP adoption decisions

Discussion

Hiring a nutritionist, collecting runoff from drylots, and testing for nutrients are practices most amenable to large operations. These practices range from 59-77% adoption rates, indicating potential for increased adoption. The perception of high cost seems to limit the adoption of hiring a nutritionist, especially for small producers who are unable to distribute the high fixed cost across as many animals. A perception of technical expertise decreases the probability of testing manure and compost for nutrients, as well as for performing yearly soil tests. The technical expertise constraint particularly impacts smaller producers for testing manure and compost, while it persists across all sizes for conducting yearly soil tests.

Both providing bedding in pens (BED) and shade in drylots (SHADE) require less technical assistance than the average practice (Table 3). This result, combined with the negative relationship between adoption and size indicates they are better suited for adoption by smaller operations, as well as operations where the feedlot represents the principal revenue stream. Results indicate Colorado respondents are more likely to adopt the practice of removing manure from drylots at least four times a year as compared to respondents from Kansas. The SURFACE model did not converge in the multivariate analysis, but the univariate analysis indicated that Colorado producers are also more likely to apply water to the surface of drylots, likely due to the dry Colorado climate.

Implications

These statistical findings should be combined with professional knowledge regarding efficacy of each BMP in terms of net ammonia emissions. Removing adoption barriers implies benefits, but not every practice costs the same to implement nor generates the same ammonia reducing benefits. Thus, the benefits and costs of increasing adoption of a BMP should be considered when prioritizing research effort and BMP subsidies. It appears that outreach and policy should prioritize practices that show both promising ammonia-reduction potential and moderate adoption rates. Specific avenues for policy may include cost-sharing, encouraging size-appropriate BMP adoption and promoting BMPs found to be profitable.

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EFFICACY OF BEST MANAGEMENT PRACTICES FOR AMMONIA REDUCTION ON FEEDLOTS AND DAIRIES

N.M. Embertson* and J.G. Davis
Colorado State University, Fort Collins, CO

ABSTRACT: Agricultural NH_3 emissions contribute to N deposition in Rocky Mountain National Park. Increased nitrate levels in alpine lakes and shifts in plant communities in the Park have been related to increasing levels of N deposition. The agricultural community is expected to voluntarily adopt Best Management Practices (BMPs) that will reduce NH_3 emissions. Our goal was to evaluate the impact of BMPs on NH_3 emissions from feedlots and dairies to provide producers with information to make choices that are both economically and environmentally sustainable. Following a thorough literature review, the most promising BMPs were tested on feedlots and dairies to measure their real-world efficacy, practicality and implementation cost. Selected BMPs were tested on 6 dairy and 6 feedlot operations in 2007 and 2008. The 12 BMPs tested were: bedding, alum application to pen surfaces, feedlot pen manure removal frequency, freestall manure removal technology, natural vs conventionally fed cattle, freestall manure removal rate, water application to drylots, composting vs stockpiling of manure, feed additives, in-pen vs out-of-pen manure stockpiling, harrowing woodchips into drylots, and natural lagoon covers. Ammonia concentration was measured from surfaces using a real-time NH_3 analyzer (Nitrolux-S, Pranalytica) with eight coincident measurements per sample location (replications varied by BMP). Results for individual BMP sampling models were analyzed using the mixed model procedure accounting for day and weather effects. Significance was evaluated at P=0.10 using lsmeans. Compost bedding in freestalls had 43% lower NH_3 concentration above the bedding surface than sand over a 30 day period (P=0.06). Harrowing wood chips into drylots tended to decrease ammonia concentration by 40% (P=0.19). Alum application to feedlot pen surfaces was not economical (~$43/head/yr), and had limited long-term effectiveness (56% reduction for 2 days). Surface emissions from naturally vs conventionally fed cattle were not different (P=0.51). Extension materials will be developed to aide feedlot and dairy managers in their management decisions.

Key Words: Ammonia, BMP, Dairy, Feedlot

Introduction

Rocky Mountain National Park has been recognized as a prominent example of the impact that nitrogen deposition can have on natural areas. Over 20 years of data have shown that wet and dry nitrogen deposition has resulted in increased soil and water N levels, which has been linked to changes in ecosystems, plant species composition, and eutrophication of surface water bodies (Fenn et al., 2003; Baron, 2006). Additionally, ammonia is known to react with atmospheric acids, such as nitric and sulfuric acid, to form fine particulate matter ($PM_{2.5}$), which is a major contributor to atmospheric smog production (Aneja et al., 1998) and has numerous detrimental human health effects such as lung and heart disease (Kaiser, 2005; Nel, 2005). Livestock operations, such as dairies and feedlots, have been credited by the State of Colorado as significant contributors to the increase in N deposition, in particular, the ammonia portion.

Nationally, and in Colorado, agriculture is listed as the largest source of atmospheric ammonia (60%), with livestock accounting for approximately 40% of total emissions (EPA, 2003). Ammonia production occurs from livestock operations when the fecal enzyme urease catalyses the hydrolysis of urea in urine, which can represent up to 70% of the total N in manure (Voorburg and Kroodsma, 1992; Todd et al., 2006). For dairy operations, model predictions show that of the ammonia emitted from dairy manure, manure application accounts for the greatest portion of volatilization (42 %), followed by housing (30 %), storage (14 %), and animals grazing pasture (14 %) (Pinder et al., 2004). For feedlots, pen surface volatilization accounts for the largest source of ammonia volatilization, which is approximately 50% (30 to 70%) of average annual feedlot ammonia emissions (Todd et al., 2008). Manure storage and land application account for the additional 50%. Land application tends to be low because most of the N has already volatilized from the manure prior to land application.

To mitigate N deposition in Rocky Mountain National Park, the State has requested that livestock operations voluntarily reduce their ammonia emissions through the adoption of Best Management Practices (**BMPs**), which are suggested to help a producer reduce their environmental impact while maintaining or improving herd production. However, while there are many suggested ammonia reduction BMPs in the literature, field testing and economic evaluation is limited. In addition, there is a need for consolidation of information about these methods to help guide producers in choosing the optimum BMPs for their operations.

The objective of our study is to minimize the negative impacts of ammonia on human health and the environment through the adoption of field-tested, effective, and economical BMPs for dairy and feedlot producers. If livestock producers are proactive and use a combination of

*Author present address: Whatcom Conservation District, Lynden, WA

various BMP reduction methods throughout their operation system, they have the potential to reduce on-farm ammonia emissions by 65-70% (Powell, 2006).

Materials and Methods

The first part of the project was to conduct a comprehensive literature review to identify BMPs that had been shown in research studies to have potential for ammonia reduction on dairy and feedlot operations. The literature review identified 12 BMPs that had potential for field success based on their effectiveness, economic viability, or ease of use. Chosen BMPs tested under field conditions included: scrape rate in freestall barns (1, 2 or 3 x/d), manure removal technology for freestall barns (scrape, automatic scrape, and vacuum), manure removal frequency of feedlot pens, composting vs. stockpiling of manure, naturally vs. conventionally fed cattle, feed additives, bedding type in freestall barns, harrowing wood chips into drylots, alum application to feedlot pen surfaces, water application to feedlot pen surfaces, in-pen vs. out-of-pen stockpiling, and natural lagoon cover.

Field demonstrations were conducted to test the selected BMPs on dairy and feedlot operations to evaluate the ammonia reduction potential, ease of use, and cost of the technologies. Field testing was vital to the validity of the BMPs, as most have only been tested in laboratory conditions, which is not a true indicator of real world conditions.

Facilities and Design. Field testing was conducted on six dairy and five feedlot operations from August to November 2007 and May to October 2008. Dairy and feedlot operations were located across Eastern Colorado and varied by type and size; however, for valid comparison, BMP testing was conducted under similar conditions at similar operations. Different BMPs require different measurement designs, but all designs were conducted using valid statistical and random models.

Sample Analysis. All ammonia measurements were conducted using a unique surface emission collector developed at Colorado State University. The system was designed to measure ammonia concentrations from pen surfaces, compost piles, lagoon surfaces, alleyways, and other locations. The device works by collecting surface emissions through 0.635 cm diameter Teflon tubing, which is protected by a PVC cap staked 10 cm above the surface. The cap is used to prevent moisture, dust, and dry deposition of gas from entering the sampling lines. The system is unique because it does not disturb the normal surface flux behavior, and thus does not alter the rate and concentration of surface emissions like other measurement devices can (i.e. flux chambers, wind tunnels, etc.). Sampling lines (8 or 16 depending on the application) collect ambient air under vacuum into a composite sampling device, which pulls air from the sampling lines at equal rates (2.2 lpm) and mixes it in a closed container. From this mixed sample, a real-time ammonia analyzer (Nitrolux-S, Pranalytic, CA) actively collects a mixed sample at a rate of 0.12 lpm. The real-time analyzer uses laser photoacoustic spectroscopy for the optical absorption of ammonia in the analyzed sample. Samples are logged every 1 to 2 minutes for analysis of surface ammonia concentration trends and variations over time. In addition, meteorological data was also collected with a real-time mobile weather station (Vantage Pro2, Davis Instruments, Hayward, CA) located near the measurement locations at the sampling site.

Statistics. Results for each BMP sampling protocol were analyzed using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC). Models accounted for day and weather effects. A repeated measures statement was included in the model if the day effect needed to be accounted for. The model (with or without repeated measures) with the lower Akaike's Information Criterion Corrected (AICC) was deemed more valid. Data were evaluated using least squares means with significant differences considered at $P < 0.10$.

Results and Discussion

Of the 12 BMPs tested in the field, complete results are only currently available for five of them. Due to seasonal constraints, management considerations, and logistics, data is still being collected for the following BMPs: water application to feedlot pen surfaces and in-pen vs. out-of-pen stockpiling. Data is still being analyzed for the following BMPs: scrape rate in freestall barns (1, 2 or 3 x/d), manure removal technology for freestall barns (scrape, automatic scrape, and vacuum), composting vs. stockpiling of manure, and natural lagoon cover.

Bedding. For compost vs. sand vs. wood shaving bedding in freestalls, results showed that when fresh (<7 days old) and well managed (restock weekly, rake weekly, surface clean daily), ammonia concentration from compost bedding (4.89 ± 0.22 ppm) was significantly higher than sand (2.25 ± 0.22 ppm) or wood shavings (3.15 ± 0.19 ppm) in the short term (<7 days; $P = 0.004$). Ammonia from sand and wood shavings did not differ under these conditions ($P = 0.82$). These results are similar to those from laboratory bedding studies (Misselbrook and Powell, 2005). However, under less managed conditions (restock bedding once every 14 days, rake weekly, surface clean daily) over a 30 day period, compost had 43% lower ammonia surface concentration (5.44 ± 1.36 ppm) than sand (9.01 ± 1.27 ppm) ($P = 0.05$). Sand bedding tended to have the most variability among beds ranging from 2 to 25 ppm, while compost had the least variation among beds (1 to 6 ppm). This is likely due to the retention of urine and feces on the surface of compost, and the infiltration of urine into sand. Over time, the sand bed becomes saturated from the bottom up and loses its ability to effectively separate the urine and feces, which is its mode of mitigation. The compost bedding on the other hand allows even incorporation of urine into the bedding upon mixing (a necessary management practice for compost beds) and stays relatively stable in concentration.

When a cost analysis was conducted, compost bedding was found to be a cost effective choice. If composting was already being practiced and necessary capital investments in composting equipment had already been made, the compost material (manure and spent feed and bedding) was readily available and required no material

input cost into the system. Sand and wood chips required regular delivery cost, fluctuation in cost, and supplier availability. Additionally, compost had the least impact on farm equipment, which can be very costly over time in manure removal equipment repairs, solid separator clean out and repair, lagoon loading of sand, and the processing of recycling of sand.

Producers typically chose sand due to its low tendency to cause health issues with cows, and choose against compost because they think it might cause an increase in udder health issues in cows. During our study, cooperating producers reported no increase in health issues with cows bedded on compost, but they did note a marked increase in somatic cell count and mastitis incidences in cows bedded on wood shavings.

Alum. Liquid application of aluminum sulfate (alum) was more effective than granular application in reducing surface ammonia concentration (56% vs. 48% reduction, respectively) within two hours of application to feedlot pen surfaces. However, unlike laboratory studies, which have found that alum reduced cumulative ammonia emissions by 98% over a 21 day period from simulated feedlot surfaces (Shi et al., 2001), we found that reductions in ammonia concentration only lasted for two days at which time they were back to baseline values. This is likely due to the mixing of the alum into the surface layer of the pens through hoof action and additional deposits of manure and urine, thus reducing the effectiveness of the alum. Additionally, at recommended amendment levels of 9000 kg/ha (Dao, 1999; Lefcourt and Meisinger, 2000; Shi et al., 2001), alum application to feedlot pen surfaces was not economical. For a 30,000 head feedlot at the necessary rate of application (every 2 days), it would cost $1.3 million per year for a 56% ammonia concentration reduction based on our results. This high cost, coupled with low profitability and the need for technical assistance explain why only 3% of producers surveyed use this practice.

Natural vs. Conventional Cattle. No significant difference was found between naturally (without feed additives, implants still given) verses conventionally (fed additives and implants given) fed cattle (P = 0.51). Economic analysis is still underway to determine if one practice is more economical based on rate of return and input cost per animal.

Manure Removal. Ammonia emissions were measured before and after scraping feedlot pens with a box-type scraper. While ammonia emissions were almost 10% higher before scraping, no significant difference was found before vs. after scraping (P = 0.51). Producer experience has demonstrated that increased pen scraping frequency reduces dust.

Wood Chips. Dairy producers typically provide some type of bedding for cows in the wet seasons. Ammonia concentrations were measured at operations that used wood chip bedding in the areas of drylot pens where cattle tended to spend a considerable (>60%) part of their day. Pens with wood chips harrowed in had up to 40% lower ammonia concentrations than areas with no wood chips (P = 0.19). While composite results were not significant, results from individual dairies were, indicating that the use of woodchips as a means to reduce ammonia concentrations above drylot pen surfaces was very effective. The wood chips acted as both a method of aerating the pen surface and as a carbon source, tying up some of the nitrogen in the manure and encouraging a composting-like process within the pen. The use of wood chips was also a good amendment to reduce pen moisture and improve cow health during wet periods.

Implications

These results demonstrate that while some BMPs may be economical on a small-scale or effective in a laboratory setting, when installed in field conditions, they are not always economical or effective. On the other hand, our findings show that there are some BMPs available that are effective, economical, and easy to use. However, individual characteristics of an operation need to be considered when choosing BMPs for dairy and feedlot operations. It is important to note that not all BMPs are viable on every operation. BMPs must be selected individually for an operation based on current management practices, BMPs already in place, operation layout, economics, and ammonia reduction goals. The results of our BMP evaluation, located on our website (www.AmmoniaBMP.info) and in outreach materials, will aid producers in choosing the BMPs appropriate for their dairy or feedlot.

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THE INFLUENCE OF LACTATION ON BOTANICAL COMPOSITION AND DIET QUALITY OF CATTLE GRAZING AT DIFFERENT STOCKING RATES IN A BUNCH GRASS PRAIRIE

A. A. Clark, T. DelCurto, S. Wyffels, C. Mueller, and G. Tschida

Eastern Oregon Agricultural Research Center, Oregon State University, Union, OR 97883

ABSTRACT: An experiment was conducted to evaluate the influence of lactation on diet quality, botanical composition, relative preference, and foraging efficiency of beef cattle grazing at different stocking densities in a bunch grass prairie. A randomized block design was used, with four (160 ha) blocks divided into four (40 ha) pastures. Four grazing treatments (stocking densities) were randomly assigned to each of the four pastures within each block; 1) control, no cattle grazing; 2) low, 0.36 animal units (AU)/ha; 3) mod, 0.72 AU/ha, and 4) high, 1.08 AU/ha for a 42 day grazing period. The research was conducted from late May through early July of 2007 and 2008. Cattle diet composition and masticate samples were collected during 20 minute grazing bouts using six ruminally cannulated cows (3 lactating cows and 3 non-lactating cows) in each experimental unit. Lactation status did not affect the percent CP or ADF in the diet averaging 10.4% and 40.6%, respectively. Lactating cow diets had lower NDF values compared to non-lactating cows ($P < 0.05$); however, the magnitude of difference may not have been biologically meaningful (61.5% vs. 62.9% NDF, respectively). Lactating cows consumed a higher percentage of Idaho fescue (33% vs. 26%, respectively; $P < 0.05$) and tended to consume less bluebunch wheatgrass (16% vs. 22%, respectively; $P= 0.08$) than non-lactating cows; however, no other differences in diet composition were noted for other grass/forb species. There were no differences ($P < 0.10$) in relative preference or foraging efficiency between lactating and non-lactating cows across the four stocking densities. In summary, our data suggests that in a bunch grass prairie foraging efficiency and relative preference are not affected by lactation status, and lactation has a minimal effect on the composition and quality of diet.

Key Words: Beef cattle, Grazing behavior, Lactation

Introduction

In order to correctly draw inferences from scientific studies it is important to understand the key biotic and abiotic factors associated with the particular study. For example a study looking at fall grazing behavior of steers in the Southwestern US may or may not help a rancher in the Midwest understand the spring grazing behavior of his pregnant cows. In grazing animal nutrition research, steers and dry cows are often used to collect dietary information due to their ease of handling. Data collected from these studies and implications made are often applied to the management of cattle as a whole (Grings et al. 2001). Hodgson and Jamieson (1981) noted that animals differing in age or physiological state may differ in respect of the digestibility of the diet selected. Grings and co-workers (2001) concluded that animals used to obtain diet samples on rangelands with diverse botanical composition should be of similar class as the animals being monitored for performance. Vanzant and co-workers (1991) conducted an experiment to determine the effects of pregnancy and early lactation on the intake, digestibility, ruminal fill and capacity, digesta dynamics, ruminal fermentation, and grazing behavior of spring-calving heifers grazing native tallgrass prairie. The authors noted physiological state had only minor effects on ruminal fermentation patterns and diet selection and no effects on digestibility, fluid dilution, distance travelled and grazing patterns throughout the day. None of the researchers above evaluated the impact of lactation on botanical composition of diet or foraging efficiency and limited information is available to compare diet quality of lactating cows versus non-lactating cows. Therefore, the objective of this study was to evaluate the influence of lactation on diet quality, botanical composition of diet, relative preference, and foraging efficiency of beef cattle grazing at different stocking densities in a bunch grass prairie.

Materials and Methods

Study Area. The study was conducted at the Zumwalt Prairie Preserve located 20 miles north of Enterprise, Oregon. The elevation of the study area ranges between 1340m and 1460m. Average annual precipitation (30 year average) is approximately 330 mm (Damiran et al. 2007). Total mean annual production for vegetation across the study area ranges from 1262 to 1928 kg/ha (Darambazar et al. 2007). The vegetation is dominated by native bunchgrasses that include Idaho fescue (*Festuca idahoensis*), prairie Junegrass (*Koeleria macrantha*), and bluebunch wheatgrass (*Pseudoroegneria spicata*), with western yarrow (Achillea millefolium), silky lupine (*Lupinus sericeus*), tall annual willowherb (Epilobium *brachycarpum*) and twin arnica (*Arnica sororia*) being the most prevalent forbs (Darambazar et al. 2007). Shrubs such as dwarf (*Rosa gymnocarpa*) and Nootka roses (*Rosa nutkana*) and common snowberry (*Symphoricarpos albus*) occur on the study site; however shrubs are a very small component in the vegetative fauna. The study area has been grazed for over 100 yr and has been used as spring/summer pasture for cattle for the past 50 years (Damiran et al. 2007).

Experimental Design. A randomized complete block design was used, with four (160 ha) blocks divided into four (40 ha) pastures. Four grazing treatments (stocking densities) were randomly assigned to each of the four pastures within each block; 1) control, no cattle grazing; 2) low, 2 heifers and 8 pair/40ha or 0.36 animal units (AU)/ha; 3) mod, 4 heifers and 16 pair/40ha or 0.72 AU/ha; and 4) high, 6 heifers and 24 pair/40ha or 1.08 AU/ha for a 42 day grazing period. The moderate stocking rate was based on traditional use common for this bunchgrass range region with low and high stocking densities reflecting 50% and 150% of the traditional stocking densities, respectively. Lactating cows used for sampling were between 110 and 150 days postpartum.

Diet Composition. Data were collected on standing crop (production by species) from late June to late July of 2006. Within each pasture 36 gridded sampling points (0.5 × 1 m, 0.5 m^2) were used to determine standing crop. All vegetation within the rectangular frame was clipped at ground level. Clipped samples were separated into live and dead materials, the latter was discarded. Live material (standing crop) was further separated by species, oven dried at 60°C, and weighed. Total standing crop of each pasture was determined by summing the aboveground biomass of all species removed from each plot within the pasture. This information was used to determine relative preference of species consumed by cattle in each treatment. Cattle dietary composition information was collected in early July from six cows (3 lactating cows and 3 non-lactating cows) in each experimental unit (40 ha pasture) using bite-count methodology similar to that described by Wickstrom et al. (1984) and Canon et al. (1987). Collections occurred in each unit after the grazing treatment was applied. Diet composition data were collected from six, (6 animals and 1 bouts/animal) 20 minute grazing trials. Each observer was assigned a cow at random for each grazing bout. Animals were accustomed to observers in close proximity and observers had prior training in plant identification. Observers used a small hand-held tape recorder to record the number of bites of each plant species consumed (Findholt et al. 2004). Relative preference was calculated for grasses and forbs by dividing percent bites consumed by percent of standing crop in the pasture.

Diet Quality. Cattle diet quality information was collected in early July from six ruminally cannulated cows (3 lactating cows and 3 non-lactating cows) in each experimental unit (40 ha pasture). Collections occurred in each unit after the grazing treatment was applied. Each cow's ruminal contents were evacuated and the ruminal wall washed with a sponge to remove remaining digesta and ruminal fluid. Cows were allowed to graze for 20 minutes and diet samples were obtained via the ruminal cannula. One rumen evacuation per cow was performed in each experimental unit. Ruminal samples were dried in a forced-air oven (55°C; 96 h) and ground to pass a 1-mm screen in a Wiley mill. Rumen samples were analyzed in duplicate for nitrogen (Leco CN-2000; Leco Corporation, St. Joseph, MI), NDF and ADF (Ankom 200 Fiber Analyzer, Ankom Co., Fairport, NY).

Foraging Efficiency. Masticate samples were collected after each 20 minute bout. Samples were dried in a forced air oven at 50°C. The dried weight was divided by 20 to determine grams consumed per minute. Grams per bite were calculated by dividing the total dried weight by the total number of bites. Bites per minute were calculated by dividing the total number of bites per grazing bout by 20.

Statistical Analysis. Data were analyzed using GLM procedures of SAS (2002) using a 2X4 factorial arrangement of treatments evaluating lactation and grazing density. Predetermined contrast statements (Steel et. al 1997) were used to determine treatment differences ($P < 0.05$). Contrast statements were 1) non-lactating vs. lactating, 2) linear, 3) quadratic, 4) cubic, 5) linear interaction, 6) quadratic interaction and 7) cubic interaction for both diet composition and quality variables.

Results and Discussion

Diet Composition and Quality. Cattle have been shown to be very selective grazers (Beck 1975; Cruz and Ganskopp 1998). Diet selection and quality of the forage consumed are affected by the chemical composition and the physical characteristics of the forage (Kothmann 1992), as well as the species composition and amount of available forage present at the site (Walburger et al. 2007). The inherent heterogeneous vegetative composition of bunch grass ecosystems allows grazing animals the opportunity to select from a variety of plant species within a pasture. In our study, grasses made up the greatest proportion of cattle diets (92% lactating, 93% non-lactating; Table 1), which agrees with other regional studies by Miller and Krueger (1976) and Walburger et al. (2007). Forb consumption was similar ($P = 0.67$; 7.5% in diet) across all treatments.

Since different plant species can vary significantly in nutritive quality (Provenza et al. 2007), stocking rate may also have an effect on diet quality and composition due to influences on the availability and structure of the vegetation present (Guevara et al. 1996). In addition, a study looking at differences in cattle diet composition due to age and sex, Grings et al. (2001) concluded that animals used to obtain diet samples on rangelands with diverse botanical composition should be of similar class as the animals being monitored for performance. In our study lactating cows consumed a higher percentage of Idaho fescue (33% vs. 26%, respectively; $P < 0.05$) and tended to consume less bluebunch wheatgrass (16% vs. 22%, respectively; $P = 0.08$) than non-lactating cows; however, no other differences in diet composition were noted for other grass/forb species.

Grasses were preferred to forbs across all treatments as determined using a relative preference index (RPI; Table 2). Relative preference values indicate a strong preference for grasses regardless of treatment and lactation status, while forbs were never preferred as a forage class in any treatment. There were no differences ($P > 0.10$) in relative preference between lactating and non-lactating cows across the four stocking densities. With no differences found in RPI values, we suggest that dry cows could be used to model lactating cows in similar diet selection and preference studies.

Physiological state has also been hypothesized to effect digestibility of the diet selected (Hodgson and Jamieson 1981). However, in a study conducted by Vanzant et al. (1991) looking at various effects of pregnancy and early lactation, the authors reported physiological state had no effects on digestibility. In our study, lactating cow diets had lower NDF values compared to non-lactating cows (Table 3; $P < 0.05$); however, the magnitude of difference may not have been biologically meaningful (61.5% vs. 62.9% NDF, respectively). Lactation status did not affect the percent CP or ADF in the diet averaging 10.4% and 40.6%, respectively. With only a slight difference observed in NDF values, biologically, dry cow's diets accurately represented lactating cow diets.

Foraging Efficiency. Foraging is the dominant activity of free-ranging ungulates (Wickstrom et al. 1984), with forage efficiency being directly related to animal performance. However, foraging efficiency is often overlooked as a component in animal nutrition research. Krysel and Hess (1993) concluded that monitoring daily grazing behavior without measuring forage intake will not provide the meaningful insight needed to understand the complex interrelationships that exist in the grazing ruminant. Measures of foraging efficiency are much more prevalent in wildlife research as opposed to domestic livestock research. Cook and co-workers (2004) found higher rates of dry matter, digestible energy, and crude protein intake in lactating cow elk than non-lactating cows being fed a high nutrition ration. The difference in intake would suggest that lactating animals would need to be used in order to obtain accurate intake and foraging efficiency measurements for cow-calf pairs. In our study, foraging efficiency of cattle decreased with increased stocking rates for both lactating and non-lactating cattle (Table 3), however, there were no differences in foraging efficiency between lactating and non-lactating across all treatments, suggesting that dry cows may be used to model foraging efficiency of lactating cows.

Implications

We interpret our data to suggest that in grazing animal nutrition/management research, lactation status has no meaningful effects on diet quality, botanical composition of diet, relative preference, and foraging efficiency. Therefore, dry cows can be used in place of lactating cows as a model for diet quality and foraging efficiency for grazing nutrition research. Likewise, dry cows can also be used to model for lactating cows in terms of botanical composition of diet/relative preference and, as a result, be used to model the ecological impact of cattle on rangeland plant communities.

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Table 1 Percent diet composition by forage class and dominant grasses and forbs consumed by non-lactating and lactating cattle grazing at 0%, low, moderate, and high stocking densities on the Zumwalt Prairie in northeast Oregon (Data averaged over 2007 and 2008).

	Non-lactating				Lactating				
	0%	Low	Mod	High	0%	Low	Mod	High	SE[2]
% Grass	**95.1**	**95.3**	**94.5**	**87.1**	**93.0**	**92.6**	**88.1**	**93.8**	**3.50**
California brome	11.8	7.1	9.5	2.7	12.3	3.8	10.1	2.6	3.63
Bluebunch wheatgrass[1]	24.3	29.5	16.7	17.6	20.0	18.1	12.7	13.1	4.70
Prairie Junegrass	2.6	1.3	0.6	0.4	2.2	1.9	0.4	0.4	0.62
Intermediate wheatgrass	8.0	0.2	0.2	0.0	10.3	1.6	0.7	0.0	3.82
Idaho fescue[1]	8.7	25.6	41.0	27.7	9.6	31.8	40.4	55.3	5.72
Sedge spp.	1.1	0.9	2.5	3.2	1.3	3.2	2.7	0.4	1.30
California oatgrass	2.0	0.1	1.5	0.0	3.0	1.9	0.0	1.5	1.26
Timothy	1.6	0.4	2.6	4.9	2.3	2.4	2.7	2.1	1.56
Kentucky bluegrass	18.6	24.3	15.5	23.1	21.2	20.3	16.4	7.7	4.80
Sandberg bluegrass	1.5	1.5	1.0	3.3	0.7	1.2	0.6	2.7	0.90
Onespike oatgrass	3.9	0.6	0.9	1.0	2.4	0.2	0.2	0.6	1.43
% Forb	**4.8**	**4.7**	**5..4**	**12.9**	**6.9**	**7.2**	**11.9**	**6.2**	**3.52**

[1] Main effect ($p<.05$),
[2] Standard Error (Pooled) (n = 4).

Table 2 Relative preference index by forage class and dominant grasses and forbs consumed by non-lactating and lactating cattle grazing at 0%, low, moderate, and high stocking densities on the Zumwalt Prairie in northeast Oregon (Data averaged over 2007 and 2008).

	Non-lactating				Lactating				
	0%	Low	Mod	High	0%	Low	Mod	High	SE[1]
% Grass	**1.8**	**1.6**	**1.6**	**1.7**	**1.8**	**1.5**	**1.5**	**1.9**	**0.13**
California brome	6.6	18.6	2.5	4.7	7.1	5.1	3.1	3.5	4.30
Bluebunch wheatgrass	1.8	1.7	1.7	1.5	1.5	1.0	1.3	1.4	0.45
Prairie Junegrass	0.6	0.3	0.1	0.1	0.5	1.7	3.9	3.5	0.75
Idaho fescue	0.4	1.4	3.8	1.7	0.5	1.7	3.9	3.5	0.75
Annual hairgrass	11.2	0.1	0.0	0.3	0.0	0.1	0.1	0.3	4.02
California oatgrass	13.9	0.6	1.9	0.0	10.2	14.2	0.0	11.4	4.02
Timothy	6.2	0.4	0.8	4.2	2.8	2.1	0.8	2.8	2.63
Kentucky bluegrass	4.0	3.9	1.7	3.3	4.5	3.4	1.8	1.1	1.02
Sandberg bluegrass	2.0	1.6	1.5	5.7	1.4	1.3	0.9	6.4	1.37
Onespike oatgrass	148.8	1.0	2.1	3.4	103.2	3.4	8.5	1.9	62.38
Intermediate wheatgrass	34.5	10.9	0.1	0.0	23.1	60.1	1.0	0.0	20.15
% Forb	**0.1**	**0.1**	**0.1**	**0.2**	**0.2**	**0.2**	**0.3**	**0.1**	**0.08**
Hoary Balsamroot	0.9	0.5	0.0	0.3	1.6	0.4	0.2	0.1	0.30
Western yarrow	0.1	0.3	0.0	0.1	0.0	0.1	0.1	0.1	0.08
Canadian milkvetch	0.0	1.4	0.3	0.4	0.1	0.9	0.7	2.3	0.85
Hawkweed spp.	2.2	0.7	0.3	0.5	3.8	0.1	0.0	2.3	1.33

[1]Standard Error (Pooled) (n = 4).

Table 3 Percent crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) of masticate samples and bites per minute (BPM), grams per minute (GPM), and grams per bite (GPB) of from non-lactating and lactating cattle grazing at 0%, low, moderate, and high stocking densities on the Zumwalt Prairie in northeast Oregon (Data averaged over 2007 and 2008).

		Non-lactating				Lactating				
		0%	Low	Mod	High	0%	Low	Mod	High	SE[2]
Diet quality										
	CP	10.3	10.3	11.0	10.9	10.5	10.1	10.2	10.0	0.5
	NDF[1]	62.6	63.6	63.1	62.2	62.1	61.0	61.4	61.5	0.9
	ADF	40.4	40.6	41.0	40..0	40.3	41.1	41.3	40.1	0.7
Foraging efficiency										
	BPM	26.0	13.3	11.0	12.7	26.7	14.2	12.7	13.0	1.8
	GPM	30.9	13.3	10.5	10.8	31.3	16.0	11.7	12.6	2.5
	GPB	1.2	1.6	1.1	1.0	1.2	1.7	1.1	1.2	0.2

[1] Main effect ($p<.05$),

[2] Standard Error (Pooled) ($n = 4$).

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COMPARISON OF FEEDING WET DISTILLERS GRAINS IN A BUNK OR ON THE GROUND TO CATTLE GRAZING NATIVE SANDHILLS WINTER RANGE

J. A. Musgrave, L. A. Stalker, M.C. Stockton and T. J. Klopfenstein

University of Nebraska, Lincoln, NE

ABSTRACT: Two-experiments determined the effects of feeding wet distillers grains with solubles (WDGS) either on the ground or in a bunk to cattle grazing native Sandhills winter range. In Experiment 1 (Exp. 1), 120 multiparous March-calving cows (536 ± 53.5 kg BW) were stratified by age and assigned to one of four treatments: WDGS fed on the ground, either three or six d/wk; or WDGS fed in a bunk either three or six d/wk. In Experiment 2 (Exp. 2), 63 March-born steer calves (201.2 ± 27.5 kg BW) were stratified by weight and assigned to one of two feeding treatments: WDGS fed in a bunk or on the ground. Both experiments were conducted at the University of Nebraska Gudmundsen Sandhills Laboratory. Exp. 1 was conducted for 90 d from Dec 1, 2007 to Mar 1, 2008, while Exp. 2 ran for 60 d from mid-Oct to mid-Dec 2008. Cows in Exp. 1 were supplemented with the daily equivalent of 0.45 kg/cow (DMB) and supplement was delivered three or six d/wk. Steers in Exp. 2 were supplemented with the daily equivalent of 1.02 kg/steer (DMB) and supplement was delivered 5 d/wk. In Exp. 1, frequency had no effect on cow BW (P = 0.55) or BCS (P = 0.27). Body condition score of cows fed in a bunk increased, while that of cows fed on the ground did not change (0.4 vs. 0.0; P = 0.01). Cows fed in a bunk lost less BW than cows fed on the ground (9.1 vs. 29.0 kg; P = 0.07). In Exp. 2, steers fed in a bunk had higher ADG than steers fed on the ground (0.29 vs. 0.20; P = 0.04). A retrospective analysis using the NRC (1996) showed a 0.14 kg/d reduction in WDGS intake would have resulted in the 0.09 kg reduction in ADG. This is the equivalent of 13% waste. Calf sale value would have to be less than \$0.81/0.45 kg to justify not feeding in a bunk based on cost of feeding in a bunk being about \$0.16/d. Frequency of delivery of WDGS did not affect animal performance. An advantage in animal performance to feeding WDGS in a bunk versus on the ground was seen in the current studies.

KEY WORDS: Wet Distillers Grains with Solubles, feeding frequency, bunk, WDGS

Introduction

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

Wet distillers grains with solubles (WDGS) have not been widely used in grazing applications. This is due, in part, to potential inefficiencies in delivery of WDGS to grazing cattle. Feeding WDGS on the ground may result in higher waste levels when compared to feeding it in a bunk, but may increase its use in practical grazing situations and increase profitability compared to bunk feeding. Therefore, the objective of this study was to compare feeding WDGS in a bunk or on the ground to grazing cattle.

Materials and Methods

Both experiments were conducted at the University of Nebraska Gudmundsen Sandhills Laboratory (GSL) near Whitman, NE according to protocol approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Cattle grazed native upland Sandhills winter range dominated by little bluestem [*Schizachyrium scoparium* (Michx.)], prairie sandreed [*Calamovilfa longifolia* (Hook.) Scribn.], sand bluestem (*Andropogon gerardii* var. *paucipilu* Hack.), switchgrass (*Panicum virgatum* L.), sand lovegrass [*Eragrostis trichodes* (Nutt.) Wood], indiangrass [*Sorghastrum nutrans* (L.) Nash] and blue grama [*Bouteloua gracilis* (H.B.K.) Lag. Ex Griffiths] (Lardy et al., 1999).

For both experiments, wet distillers grains were obtained from an ethanol production facility (Standard Ethanol, LLC; Madrid, NE) and transported about 179 km to GSL. The distillers grains was purchased in September each year and stored in a bunker fashioned from large round bales of meadow hay arranged in a "U" shape and covered with plastic until initiation of the experiment, according to methods outlined by Erickson et al. (2008).

In Exp. 1, 120 multiparous March-calving cows (536±53.5 kg BW) were stratified by age and assigned randomly to one of eight pastures. Pastures were then assigned randomly to treatment. Treatments were arranged as a 2 X 2 factorial in a completely randomized design as follows: WDGS fed on the ground, either three or six d/wk; or WDGS fed in a bunk either three or six d/wk. The experiment was

conducted for 90 d from Dec 1, 2007 to Mar 1, 2008, Cows were supplemented with the daily equivalent of 0.45 kg/cow (DMB) WDGS, delivered on Monday, Wednesday and Friday to cattle in the three d/wk treatment and Monday through Saturday to cattle in the six d/wk treatment. Cattle continuously grazed the same pasture throughout the experiment. Cow BW and BCS were measured upon initiation and completion of the 60-d feeding period. Weights were taken on a single day and cows were not limited fed prior to weighing.

Experiment 1 data were analyzed using MIXED procedures (SAS Inst. Inc., Cary, NC) and the model included the effects of feeding method, frequency of WDGS delivery and their interaction. Pasture was used as the experimental unit. Differences were considered significant when *P*-values were < 0.10.

In Exp. 2, 63 March-born steer calves (201.2 ± 27.5 kg BW) were stratified by weight and assigned to one of two feeding treatments: WDGS fed in a bunk or on the ground. Steers in Exp. 2 were supplemented with the daily equivalent of 1.02 kg/steer (DMB) and supplement was delivered five d/wk. The experiment was conducted for 62 d from October 14, 2008 to December 15, 2008. A total of four experimental pastures were used resulting in two observations per treatment. Steers continuously grazed the same pasture throughout the experiment. Steer BW was recorded on two consecutive days at the initiation and completion of the feeding period. Calves were not limit fed prior to weighing.

Experiment 2 data were analyzed as an unstructured treatment arrangement in a completely randomized design using MIXED procedures (SAS Inst. Inc., Cary, NC). The model included the effect of feeding method. Pasture was used as the experimental unit. Differences were considered significant when *P*-values were < 0.10.

Results

In Exp. 1, there were no frequency by method interactions ($P > 0.10$). Frequency had no effect on cow BW ($P = 0.55$) or BCS ($P = 0.27$). Body condition score of cows fed in a bunk increased, while that of cows fed on the ground did not change (0.4 vs. 0.0; $P = 0.01$; Table 1). Cows fed in a bunk lost less BW than cows fed on the ground (9.1 vs. 29.0 kg; $P = 0.07$; Table 1). Previous research as GSL has demonstrated 0.14 kg/d of supplemental crude protein to be sufficient to maintain BCS of spring-calving cows during the winter (Hollingsworth-Jenkins et al., 1996). In this experiment feeding WDGS in a bunk at an equivalent crude protein level resulted in a slight increase in BCS. This may be a result of the energy content of WDGS. While better performance was achieved by feeding in a bunk, this experiment demonstrated WDGS is a viable supplement for cows grazing winter range.

In Exp. 2, steers fed in a bunk had higher ADG than steers fed on the ground (0.29 vs. 0.20; $P = 0.04$; Table 2). The NRC (1996) was used to retrospectively calculate the WDGS intake difference between treatments. For steers fed in a bunk, 0.14 kg/d reduction in WDGS intake would have resulted in a 0.09 kg reduction in ADG. It was therefore assumed 0.14 kg/d of the WDGS offered to steers fed on the ground was wasted. This is the equivalent of 13% waste. Because steers in this experiment were gaining BW at a relatively modest rate, even a slight reduction in WDGS intake resulted in a relatively large decrease in ADG. If the steers were being fed to achieve relatively rapid BW increases and waste of WDGS remained constant than the relative difference in ADG between cattle fed in a bunk versus on the ground would be expected to be less than what was observed in this study

An economic analysis was conducted on Exp. 2 (Table 3). This analysis was based on the value of the average difference in weight gained between steers fed WDGS in a bunk or on the ground. Calf sale value would have to be less than \$0.81/0.45 kg to justify not feeding in a bunk based on cost of feeding in a bunk being about \$0.16/d. The cost of \$0.16/d was derived from the purchase of a commercial (Werk Weld Inc., Armour, SD) feed bunk, assuming full capacity of 40 h. Bunk cost of \$973.65 included a onetime delivery charge with a three year pay back and 60 days of use per year at an interest rate of about 9.5%. Bunk cost for individual producers will vary as will calf value necessary to justify bunk feeding (Table 3).

In conclusion, frequency of delivery of WDGS did not affect animal performance. An advantage in animal performance to feeding WDGS in a bunk versus on the ground was seen in the current studies.

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Table 1. Change in body weight (BW) and body condition score (BCS) of cow fed WDGS on the ground or in a bunk (Exp. 1)

	Bunk	*Ground*	*SEM*	*P-value*
BCS Change	0.43	0	0.068	0.01
Weight Change	-9.1	-29.0	5.6	0.07

Table 2. Performance of steers fed WDGS on the ground or in a bunk (Exp. 2)

	Bunk	*Ground*	*SEM*	*P-value*
Initial BW	199.7	202.7	5.0	0.67
Final BW	218.0	215.4	5.0	0.71
ADG	0.29	0.20	0.03	0.04

Table 3. Value of the difference in ADG between steers fed WDGS in a bunk or on the ground (Exp.2).

Value of 0.45 k live weight	Value of 0.09 kg/d weight difference
$0.80	$0.159
$0.85	$0.169
$0.90	$0.179
$0.95	$0.189
$1.00	$0.198
$1.05	$0.208
$1.10	$0.218
$1.15	$0.228
$1.20	$0.238
$1.25	$0.248
$1.30	$0.258
$1.35	$0.268
$1.40	$0.278
$1.45	$0.288
$1.50	$0.298

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DEVELOPMENT AND IMPLEMENTATION OF A VERTICALLY-INTEGRATED BEEF CATTLE DATA COLLECTION SYSTEM

D.J. Drake†, A.L. Van Eenennaam†, and J.D. Lowe‡
†University of California, Davis, CA and ‡Midwest MicroSystems, Lincoln, NE

ABSTRACT: Breeding decisions made by cow-calf producers may be suboptimal on a chain-wide basis if they never receive performance data from the feedlot or harvest sectors. However, the segmented nature of the beef industry presents challenges to sharing data collected by each of the supply chain members. We developed a data collection system using commercially available computer software to track the performance of individual animals from birth through subsequent production phases to the final carcass value. Both the University of California cow-calf herd and commercial cooperator herds participated in this project. The key to linking records from each phase was a radio frequency identification (RFID) ear tag assigned to each animal in the cow-calf herd. Sector-specific software developed by Midwest MicroSystems L.L.C. including Cow Sense® for ranch data, MARS for feedlot data, and Beef STAR® for harvest data collection were used for the real time transmission of field data to off-site "office" computers. A small commercial processing facility cooperated in the program and collected harvest data using a handheld device. After initial training focused on transferring identification from pre- to post harvest, carcass data were routinely obtained from all cattle processed. ID transfer at a small processing facility was simplified by relatively slow chain speeds. Data collected by collaborators were transmitted to a central server where they were connected with other research data (e.g. DNA genotypes) in a Microsoft® Access database. Data correction privileges were only extended to field collaborators. Data consumers received "read only" permission levels, thereby maintaining a single source of data control and integrity. Benefits were 1) minimal disruption or changes to individual sector data collection methods and labor, 2) integration of data across sectors, 3) data integrity and security, 4) returning performance data to cow-calf producers to facilitate more informed selection decisions, and 5) development of a comprehensive dataset for research.

Key Words: beef cattle, integrated data

Introduction

Cow-calf producers rarely obtain feedlot performance or carcass quality information on the calves they produce. As a result there is little opportunity or incentive for them to make genetic improvement in traits that are of importance to the feedlot and processing sectors, and consumers. Some producers have opted to join integrated beef production programs whereby they receive carcass data back from the processor, and receive premiums for the production of carcasses that achieve certain quality targets. Such programs provide both the information and the market incentive for producers to include carcass quality traits in their selection criteria. However, feedback requires a system that can integrate records coming from the different sectors of the beef supply chain. The increasing use of unique individual animal radio frequency identification (**RFID**) devices as a part of the National Animal Identification System (NAIS) offers an opportunity to introduce beneficial feedback to the supply chain.

The value of comprehensive data collection can be further increased through the simultaneous use of DNA markers to resolve the paternity of offspring produced in multisire breeding pastures, thus enabling on-ranch genetic evaluations (Dodds et al. 2005; Pollak, 2005; Van Eenennaam et al., 2007). In New Zealand over 20% the ram, and 30% of the deer breeding industry are now using DNA-enabled commercial ranch sire evaluations (McEwan, 2007). Ideally, information from each sector would be made available to breeders to use in selection decisions that optimize production to meet consumer demand (Figure 1).

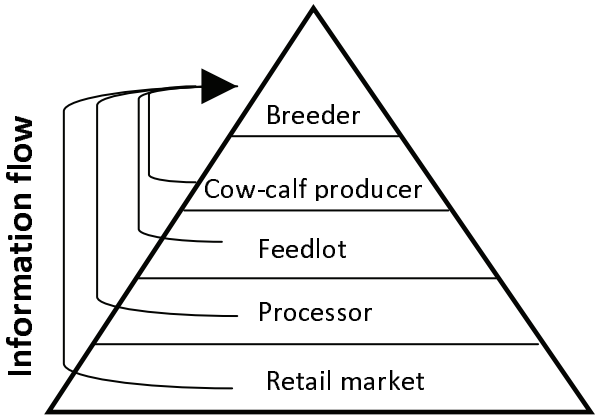

Figure 1. The greatest value of a vertically-integrated data collection system is obtained when breeding decisions are optimized for the entire chain (Modified from McEwan, 2007).

McEwan (2007) proposed that the widespread adoption of DNA technologies will depend on reducing the costs associated with sampling, DNA extraction, reporting and integrating the data into genetic evaluations. In New Zealand, DNA collection is being linked to electronic tags, allowing automation of subsequent steps and return of data to genetic evaluation entities. If DNA information is going to become widely adopted and used for genetic evaluation purposes in the U.S., it is likely that such approaches will also need to be implemented here. Currently, different sectors of the beef cattle industry are adopting computer technology for their own needs, and there is little

integration between sectors, limiting both proactive exchange of information for management as well as post hoc evaluation of data for decision-making purposes. Effective traceback related to food safety or animal health issues is also limited by industry fragmentation. Development and implementation of voluntary integrated animal identification systems that simultaneously provide assistance in management as well as biosecurity features would be useful. We developed a data collection system using commercially-available computer software and RFIDs to track the performance of individual cattle from birth through subsequent production phases to final carcass data measurements.

Material and Methods

Cow and calf herds owned by the University of California Animal Science department and three commercial ranchers were involved with this project. The UC herd consisted of about 300 breeding females that calve in the fall (October). Steers and cull heifers were sent to the campus feedlot about 100 km away for finishing. Harvest-ready cattle were transported 225 km to a commercial harvest facility for processing. Commercial cow calf ranches were part of an integrated beef production program with a feedlot and harvest facility about 700 km away. Commercial ranch A consisted of a fall calving (September) herd of about 500 breeding females and a spring calving (January) herd of about 300. Commercial ranch B consisted of a fall calving (October) herd of about 300, and a spring calving herd (February) of about 200. Ranch C was spring calving (January) with about 200 breeding females. The UC herd and feedlot had a history of computerized performance records primarily using spreadsheets (Microsoft® Excel). The commercial herds had no prior experience with computerized records with the exception of ranch B, which had used a database program (CowBoss) to a limited extent. Prior to this project, carcass grading reports were provided on UC cattle via fax from the harvest facility. The commercial ranches received printed individual carcass data without individual animal identification, and some summary carcass data statistics.

All ranches on this project used multisire breeding pastures of 50 to 100 breeding females with ratios of approximately 25 females per bull for fixed durations of 45 to 90 days, depending upon the ranch. Calving records consisted of birth dates and dam identification, typically recorded within 1-2 days of birth in pocket-sized cattle record books. At birth, calves were identified with individual numbers and ear tags were applied. During the course of this project these records were then transferred to Cow Sense® herd management software (Midwest MicroSystems, Lincoln, NE). RFID ear tags were applied at weaning, at which time hair samples from calves from UC and commercial ranch A for 3 calf crops were collected for DNA-based paternity assignment (Van Eenennaam et al., 2007; Van Eenennaam et al., 2009).

During the feedlot phase, UC calves were weighed upon entry and at 30 day intervals. Feedlot data was collected using the Measurement and Analysis Research System (**MARS**), a computer program for multiple measures of cattle (Midwest MicroSystems). Cattle were harvested based on visual estimates of finish and shipped in groups of approximately 20 head for harvest and carcass data collection by a USDA grader. Carcass data were collected at the processor using a handheld device (PSION Workabout Pro) similar to those used by overnight delivery services, and Beef STAR® Processor software (Midwest MicroSystems). Beef STAR® is designed specifically for carcass data entry and transmission.

The calves from the three commercial collaborators were fed in a single group for each ranch and harvested in a single day. Harvest criteria varied depending on the needs of the processor. No individual feedlot performance information was available on these animals. Carcass data were collected at the large commercial processing plant by a company grader and provided to the collaborators in spreadsheet format. This data were then brought into Cow Sense® using the import tool and matched up with cow-calf sector data using RFID.

Results

Conceptually the data management system that is in place for the UC Davis cow-calf herd is composed of several distinct operations (Figure 2). Various sectors (users) collect data with commercially available software designed for their specific needs. This data are exchanged via remote computer servers (Figure 2; dotted lines) to a central office computer (Figure 2; large bold arrow) that resides in the UC Davis Animal Science department. The users can be physically separated with the data exchange being made via the internet through the Beef STAR® software program. At the central location or server, databases from the sectors are connected in a generic Microsoft® Access database. Data flows through Beef STAR® back to Cow Sense® herd management software to provide the herd manager with information on calf performance in the feedlot and at harvest. Data integration also enables development of on-ranch EPDs for the herd bulls on each ranch (Van Eenennaam et al. 2008, Van Eenennaam et al. 2009).

A specific example illustrates data flow (Figure 3). Calving data were collected at the cow/calf ranch in Cow Sense®. Additional data at that sector were collected until the calves were shipped to the next sector, the feedlot. At this time, data were sent from Cow Sense® to the central server allowing feedlot personnel access to data as calves were incoming and processed. Feedlot-specific data such as pens, rations, and in-weights associated with the RFIDs were recorded chute-side in MARS, which is their sector-specific software program, then transmitted to the central server. The processor and/or USDA personnel collected carcass data in the cooler with an electronic ID reading device (Psion). These data were transmitted electronically via Beef STAR® back to the central server where the data were stored. The commercial ranches received carcass data electronically in spreadsheets from the processor, and these were downloaded into Cow Sense® linked by the animal's RFID.

If additional research data (e.g., ultrasound scans or DNA genotyping results) were collected beyond that

identified in the commercially available software, the information was linked via the RFID number and included in the central Access database (Figure 2).

At cattle handling facilities where data collection occurred, whenever it was possible, wireless connections were established so data flowed directly into office desktop computers rather than onto portable computers carrying copies of the database. This networking improved data security and integrity. Additional database security was provided with remote location backup of databases and read only privileges for researchers accessing the central database.

Discussion

Cow and calf sector. Collection of calving data was the most problematic for a variety of reasons. Unlike the feedlot and harvest where large numbers of cattle were processed at one time, calving is ongoing over several weeks or months. Frequently, when ear tagging neonatal calves, behavioral difficulties were encountered with their mothers. Cold or wet weather also often made the collection of detailed records difficult. Dam identification is important for improving production, but it can be difficult to read or remember the identification of the mother when working with neonatal calves. All cow/calf producers continued to record calving data in "red books" or their equivalent despite the opportunity to use handheld electronic devices (PDAs). In some cases, lists of periparturient cows were developed from the computerized records to facilitate accurate recording of dam identification. The field forms helped prevent errors reading dam ear tags and also provided a cross check for dams already recorded as calved. Data entry into Cow Sense®, the cow/calf database, was facilitated by data entry options in Cow Sense®, including importation of interim spreadsheet calving data.

Pre-weaning management procedures such as vaccinations were generally enhanced by having computerized records. Weaning weights were obtained with electronic scales interfaced with Cow Sense®. RFIDs were typically applied at weaning. RFID application and weighing were completed in conjunction with other standard cattle management activities.

Feedlot sector. Ownership of UC calves was retained through the UC feedlot, and data collected at the cow/calf ranch was accessible on receipt of the calves at the feedlot. This facilitated assignment of dietary, production and/or experimental groups. Physically, cow/calf data in Cow Sense® was transmitted via Beef STAR® to the feedlot to be utilized real-time for incoming calves. RFIDs were used as the linking field between cow calf and feedlot performance. Intensive feedlot data were collected in MARS. The most common measurement was repeated weighing at 30-day intervals. Commercial ranch calves did not have feedlot data collection as they were sold to the vertically integrated feedlot/processor.

Harvest sector. Carcass data on UC cattle were collected by a processor employee operating a handheld electronic device using Beef STAR® software, working with a USDA grader. RFIDs were transferred from the live animal to the carcass during harvest, and were scanned by the handheld device. The USDA grader orally communicated to the employee, who entered the information directly into Beef STAR®. This process was rapidly adopted by processor personnel following a single instructional session on how to use the handheld device and Beef STAR® software. The integrated RFID scanner in the device simplified reading RFIDs and data collection.

When carcass data collections were finished, processor secretarial employees placed the handheld data collection device in a cradle, establishing a link with their computer. With a brief 15 minute training period, the employee transmitted the data routinely from that device via the Beef STAR® software program and the internet to the central database at the Department of Animal Science.

Implications

This integrated data collection system was used to obtain cow-calf and carcass data from three UC Davis calf crops, and carcass data from three seasons of calves harvested from one of the commercial ranches. Additionally, calves and potential sires were genotyped using a SNP-based parentage panel. We are now using the information in the central database to develop on-ranch EPDs. Now that the system is operational, the central database will expand with time. Eventually we hope to develop a comprehensive database of DNA and phenotypes that will prove useful for the future validation of DNA-marker tests and whole genome-based genetic predictions.

Acknowledgements

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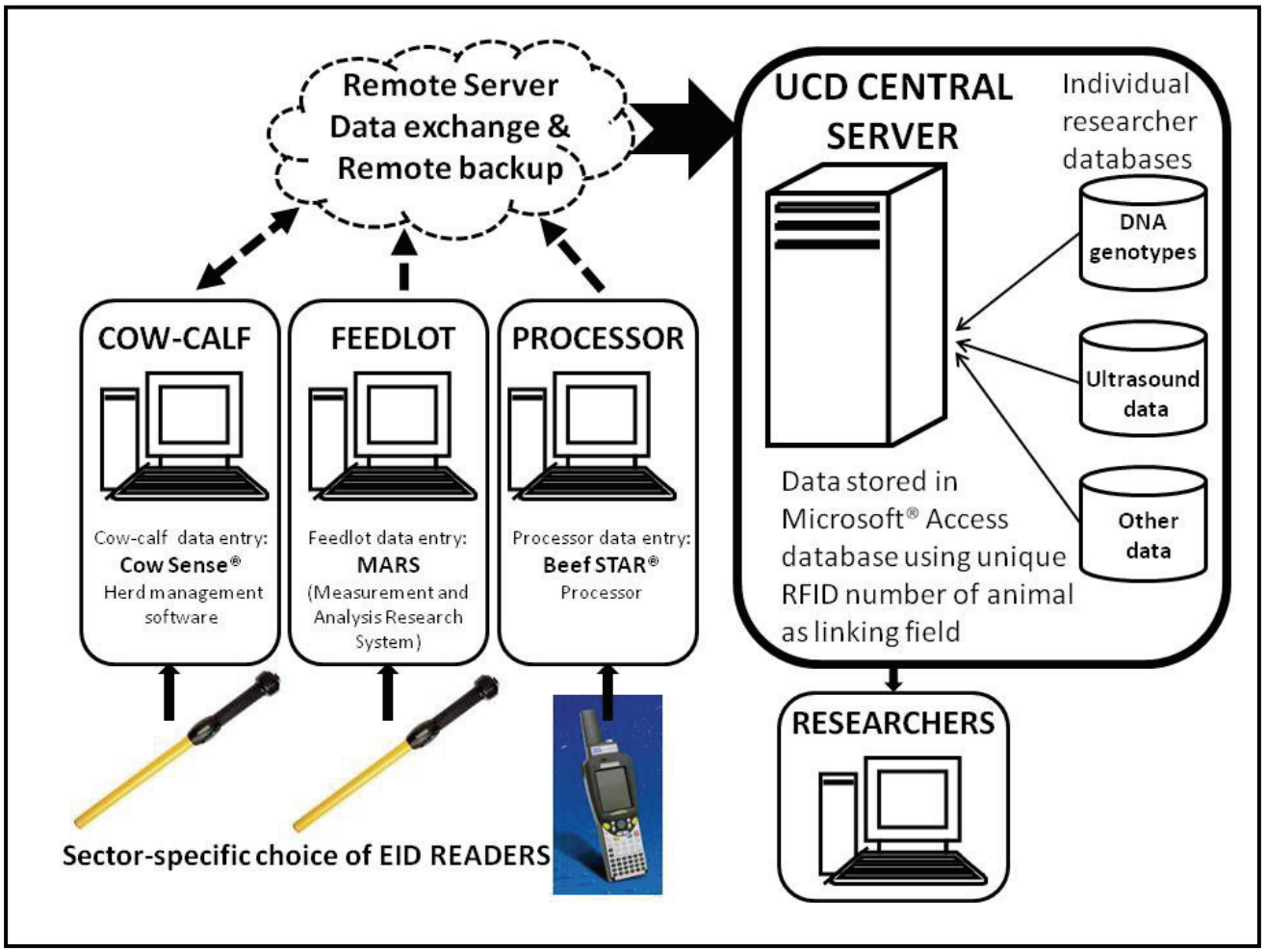

Figure 2. Schematic representation of the integrated data collection and management system. Individual users (denoted with solid-lined boxes) enter data from their facilities using combinations of office, facilities computers or handheld devices into commercially available software (Cow Sense®, MARS, Beef STAR®; Midwest MicroSystems, Lincoln, NE) designed for sector-specific data. Sole authority, capability and responsibility for data entry resides at these sites. Data are exchanged from the various data collection sectors via remote servers (denoted with dotted lines) to the central database files (thick solid line). Individual researcher databases are integrated with the sector data. Researchers and collaborators can access data with password permission, but they cannot enter or edit data on the central server.

COW-CALF

RFID	840003003747887
Calf ID	7002
Sex	H
Birth Date	9/12/2007
Birth Weight	62
Cow Age	2
Bull ID	1AN1105
Bull Breed	Angus
Wean Wt	574

FEEDLOT

RFID	840003003747887
Feeding In Date	6/12/2008
Feeding In Wt	570
Final Wt Date	12/2/2008
Final Wt	1075
Feeding Days	172
ADG Feedlot	2.94

PROCESSOR

RFID	840003003747887
Hot Wt	615
Carcass Mat.	A
Marbling Score	SM30
Quality Grade	Ch-
Marbling No.	5.3
Final YG	3.1
Carcass Backfat	0.44
Carcass REA	11.2

DNA DATABASE

RFID	840003003747887
DNABARCODE	840000000197168
CAPN316	C/G
CAPN4751	C/T
CAPN530	A/G
UOGCAST1	C/G
WSU_CALPAS	C/T
AY761135	A/A

Figure 3. Representative data on a randomly-selected calf from the 2007 UC Davis cow-calf herd. This example shows data derived from the cow-calf sector, the feedlot, the processor, and DNA genotype data from an individual researcher database. Many more data fields are included in the actual database, this subsample is just for illustrative purposes. Double-headed arrows represent two-way exchange.

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INFLUENCE OF PEN-SHADE ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF BULLS NATURALLY EXPOSED LONG TIME TO HIGH TEMPERATURE

.

R. Barajas [1]*, B. J. Cervantes[1, 2], M.A. Espino[1, 3], F. Juarez[1], J. A. Romo[1] and E. A. Velazquez[1]
[1] FMVZ-Universidad Autónoma de Sinaloa, México. [2] Ganadera Los migueles, S.A. de C.V. [3] Tecnologia de Máxima Producción, S.A. de C.V.
E-mail: rubar@uas.uasnet.mx

ABSTRACT: With the objective of determine the influence of pen-shade on feedlot performance and carcass characteristics of bulls naturally exposed long time to high temperature. Sixty bull-calves (BW = 216 ± 8.7 kg) were used in a 248-days length feedlot experiment. In accordance to a randomized complete blocks design, in groups of five the calves were assigned to be placed in pens providing or not shade. Shade increased (P = .04) and enhanced (P = .03) in 8.8% average daily gain and 6.14% feed/gain ratio in relationship with unshaded cattle. Dry matter intake was not affected by shade (P = .50). The absence of shade in pen decreased (P = .04) in 5% and 7% respectively retained NEm and NEg of the diet, compared with bulls placed in shaded pens. The usage efficiency of dietary-NEm (Observed/expected NEm) was 4.5% higher (P = .04) in shade protected cattle. Hot weather conditions increased 12% NEm expenditures in cattle located in shade deprived-pens, while the energy cost for bulls sited in shaded pens was 8%. Shade increased (P < .01) 6.7% carcass weight and enhanced (P = .02) 1.15% hot carcass dressing. KPH-fat and Back thickness fat were higher (P = .05) in carcass from animal placed under shade. Marbling and Rib eye area were not affected (P > .40) by treatments. Blood cortisol measured at death time was 55% higher (P < .01) in bulls that were deprived of shade along experiment.
It is concluded that despite of shade for itself is not enough to alleviate all detrimental effects of heat stress, helps cattle to cope adverse hot weather conditions and its benefice is reflected on feedlot performance.

Key words: Hot weather, Bulls, Feedlot-performance.

Introduction

When air temperature is increased away from its thermoneutral zone the bovines suffer heat stress (NRC, 2000; Beatty et al., 2006), hot weather has adverse effects on the performance livestock (Hahn, 1999), and enlarge beef cattle requirements of energy for maintenance (Ames et al., 1980; Morrison, 1983; NRC, 2000). Solar radiation influence greatly heat load (Mader et al., 2006) and alter the ability of the animal to maintain thermal balance (Brosh et al., 1998). Furthermore, high relative humidity boosts cattle heat stress (Blackshaw and Blackshaw, 1994). The use of shade inside of feedlot-pens is an alternative practice to alleviate partially the heat stress of cattle (Garret et al., 1962; Mader et al., 1999). The benefit of shade has been questioned for use in temperate regions (Boren et al., 1961; Bond and Laster, 1975; Mader et al., 1999). The experiments conducted under strong hot weather of southern USA, shown advantage of use of shade on feedlot performance; however length of feedlot period has been not long with duration of 54, 84 and 131 days for experiments of Ittner and Kelly (1951), Garrett et al. (1960) and Mitlohener et al. (2001), respectively. Based in results of three experiments, conducted in Nebraska during 76 to 81 days Mader et al. (1999), concludes that once cattle are acclimated or hot condition subside, compensation by unshaded cattle offsets much of the initial benefits of providing shade. There is little information about gain of shade for cattle naturally exposed for long time to hot environment on feedlot performance. The state of Sinaloa, localized at Northwest of Mexico in a dry tropical weather, is the most important region dedicate to feedlot industry in Mexico. In this area, historically mean temperature oscillates between 24 to 30ºC from April to November (INEGI, 2009), so that the knowledge of potential utility of shade for cattle exposed long time to hot weather becomes important for feedlot industry in this region.

This research was conducted with the objective of determine the influence of shade in pen on feedlot performance and carcass characteristics of bulls naturally exposed long time to high temperature.

Material and Methods

Location

The experiment was conducted during 248 days from March to November, 2008 at Experimental Station for Beef Cattle in Dry Tropic Weather of the Universidad Autonoma de Sinaloa. The research facilities are located at Feedlot Yard Ganadera Los Migueles, S.A. de C.V. in Culiacan, Sinaloa situated in Northwest Mexico (24º 51' N. and 107º 26' W. ; 57 m o.m.s.l.; mean temperature 25 ºC, and 645 mm annual rainfall).

Animals Management

Animals used in the experiment were managed according to the recommended guidelines in *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1988).

Sixty bull-calves (BW = 216 ± 8.7 kg) proximately 50% *Bos indicus* with remainder of Simmental, Angus Charolais, and Brown Swiss in undeterminate proportion were used. Calves were weighed identified with a numbered ear tag, implanted (Component TES with Tylan®;ELANCO Co.), vaccinated to prevent infections by *Manheimia sp*. (One Shoot®; Pfizer Ltd.), *Clostridium* and *Haemophilus somnus* (Ultrabac-Somnobact®; Pfizer), dewormed (Albendaphorte®; Lab. Salud y Bienestar), and injected with vitamins A, D and E (ADEphorte®; Lab. Salud y Bienestar). Groups of five calves were randomly placed in 12 pens (6 x 12 m), each of them fitted with a 2.4 m feed bunk and 0.6 m waterer. Animals had *ad libitum* access to feed and water.

Treatments

In accordance to a randomized complete blocks design described by Hicks (1973), in groups of five the calves were assigned to be placed in pens providing or not shade. Pens (6 x 12 m) were ground floor each of them fitted with a 2.4 m feed bunk and 0.6 m waterer. Shade (six pens) was provided with five metallic-layers (0.9 x 4 m) fitted 3.6 m over ground, provided pen space and shade area by head were 14.4 m^2 and 3.6 m^2, respectively. Pen in not shade treatments had the same dimensions, but without ceiling.

Experimental procedure

Diet composition is presented in Table 1. Cattle had *ad libitum* access to the diets that were offered once daily (1600 h), had *ad libitum* access to clean water. Feed intake was measured as feed offered minus weekly refusals. Feed samples (4 kg) were collected weekly directly from mixer wagon, oven dried (105 °C for 24 h), and dry matter intake calculated. Thirty three days before slaughter date, all diets were supplemented with 6 mg/kg of the beta-adrenergic zilpaterol chloride (Zilmax®; Intervet), three days previously to slaughter, zilpaterol was take out of the diet. Animals were weighed on days 1, 28 and at the end of the experiment.

Carcass Measurements

Upon complete the feedlot experiment time, the bulls were sacrificed in a Federal Inspection Type slaughter house. Hot carcass weights were recorded, and after 24 hours chilling period in a cold room (2 ºC), left carcass side longissimus muscle was cross sectioned between the 12th and 13th rib, back fat (cm) and longissimus muscle area **(LMA**) was measured by direct grid reading, marbling score and percentage of KPH-fat was visually estimated (USDA, 1996). Meat pH was measured in *pectoralis profundus* muscle using a pH-meter fitted with a penetration electrode (HI8314 membrane pH-meter; Hanna Instruments).

Serum determinations:

At death time, blood samples were taken using plain glass vacuum tubes (Vacutainer 6431; Becton Dickinson, Rutherford, NJ). Serum was obtained for cortisol, measurements. Serum cortisol was determined by radioimmunoassay using antibody-coated tubes (Diagnostic Products Corp., Los Angeles, CA).

Table 1. Composition of basal diets used in feedlot performance experiment

Ingredients	Diets			
	Receiving	Starting	Growing	Finishing
Corn straw	18.29	8.89	-	13.18
Corn silage (few grain)	51.48	38.52	23.37	-
Ground corn	-	22.22	44.15	57.79
Corn DDG	14.54	15.45	14.01	12.44
Soybean meal	6.10	3.56	-	-
Sugar cane molasses	5.08	7.40	11.42	10.98
Tallow	-	-	1.67	2.79
Ganabuffer [1]	1.13	0.99	0.85	-
Ganamin Total	3.39	2.96	2.54	2.82
Total	100%	100%	100%	100%
	Calculated Analyses (DM basis) [2]			
DM, %	44.29	50.02	59.12	88.64
CP, %	14.99	14.40	14.00	13.79
NEm, Mcal/kg	1.452	1.689	1.952	2.045
NEg, Mcal/kg	0.873	1.079	1.305	1.379

[1] Ganabuffer® (Buffer agent blend) and Ganamin Total ® (Vitamins and mineral premix) containing 25 g of sodium-monensin from Rumensin 200 ® (Elanco), and Ganabuffer ® (Buffering agents blend), are trademarks (Técnica Mineral Pecuaria, S.A. de C.V.; Guadalajara, Jal., México).

[2] Calculated from tabular values (NRC, 2000).

Statistical Analysis

Performance and serum data was analyzed as a randomized complete blocks design (Hicks, 1973), considering each pen as the experimental unit. General AOV/AOCV procedure of Statistix® 8 program (Analytical Software, Tallahassee, FL) was used to perform the analyses, and *P*-value for F-test was obtained.

Results and Discussion

The influence of shade on feedlot-performance of bulls is shown in table 2. Shade increased (P = .04) and enhanced (P = .03) in 8.8% average daily gain and 6.14% feed/gain ratio in relationship with unshaded cattle. This result is agree with improvement of feedlot performance observed in other experiments conducted under heat weather condition (Garret *et al*., 1960; Mitlohener *et al.*, 2001).

Dry matter intake was not affected by shade (P = .50). The lack of influence of shade on food intake is in concordance with observed in several experiments (Bond and Laster, 1975; Brosh et al., 1998; Mader et al., 1999).

The absence of shade in pen decreased (P = .04) in 5% and 7% respectively retained NEm and NEg of the diet, compared with bulls placed in shaded pens. The usage efficiency of dietary-NEm (Observed/expected NEm) was 4.5% higher (P = .04) in shade protected cattle. Hot weather conditions increases 12% NEm expenditures in cattle located in shade deprived-pens, while the energy cost for bulls sited in shaded pens was 8%. This results is interpreted that if shade reduce partially solar radiant energy incomes of cattle and is know that solar radiation influence greatly heat load (Mader et al., 2006), the bulls placed in shaded pens had a lower hot load than cattle shade-deprived, and have less adverse condition to dissipate excessive hot load to environment. Mean air temperature in the place that experiment was performed across March to November was 26.2 ºC and mean maxima air temperature was 33.9 ºC (CNA, 2009), showing that cattle involved in this research was permanently under heat-stress condition, both placed in shaded and unshaded pen bulls. The lost 8% in usage efficiency of dietary NEm exhibited by shade protected cattle is consequence of that, taken account that heat stress enlarge beef cattle requirements of energy for maintenance (Ames et al., 1980; Morrison, 1983; NRC, 2000). These results suggest that shade can help cattle to save up to 50% of NEm expenditures, in view that unprotected bulls expended 12% extra of NEm for maintenance.

Effect of shade on carcass characteristics is presented in Table 3. Shade increased (P < .01) 6.7% carcass weight and enhanced (P = .02) 1.15% hot carcass dressing. KPH-fat and Back thickness fat were higher (P = .05) in carcass from animal placed under shade. Marbling and Rib eye area were not affected (P > .40) by treatments. Blood cortisol measured at death time was 55% higher (P < .01) in bulls that were deprived of shade along experiment.

Implications

Results suggest that despite of shade for it self is not enough to alleviate all detrimental effects of heat stress, helps cattle to cope adverse hot weather conditions and its benefit is reflected on feedlot performance.

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Table 2. Influence of shade in pen on feedlot performance of bulls

Variable	Treatments		SEM [1]	P-value
	Shade	No Shade		
Bull-calves, n [2]	29	29		
Pen replicates, n	6	6		
Days in trial, n [3]	211	211	10.40	
Initial weight, kg	216.73	215.67	8.68	.93
Ending weight, kg	494.81	471.68	5.40	.04
Average daily gain, kg/day	1.341	1.232	.03	.03
Dry matter intake, kg/day	8.841	8.319	.15	.50
Feed/gain, kg/kg	6.357	6.773	.09	.03
Dietary net energy observed, Mcal/kg				
Maintenance	1.794	1.706	.02	.04
Gain	1.163	1.085	.02	.04
Observed / expected net energy				
Maintenance	0.92	0.88	.02	.04
Gain	0.90	0.84	.02	.04

[1] Standard error of the mean
[2] Two animals died at 162nd and 223rd day by heat shock
[3] Animals of the heavier block were sacrificed at day 165 and the lighter block was sacrificed at day 248.

Table 3. Influence of shade in pen on carcass characteristics, meat pH, and blood concentration of cortisol at death time of fattening bulls.

Variable	Treatments		SEM	P-value [1]
	Shade	No Shade		
Bull-calves, n [2]	29	29		
Hot carcass weight, kg	311.57	291.96	3.10	< .01
Carcass dressing, %	63.01	61.86	.27	.02
Back fat thickness, cm	0.79	0.67	.04	.05
Kidney, pelvic and heart fat, %	2.09	1.87	.08	.05
Marbling [3]	454	452	9.48	.88
Rib eye area, cm^2	81.12	79.47	1.46	.45
Muscle pH	6.19	6.23	.04	.51
Cortisol at death time, µg/dL	3.00	4.65	0.24	< .01

[1] Standard error of the mean
[2] Two animals died at 162nd and 223rd day by heat shock
[3] Code: traces = 300; slight = 400; small = 500; modest = 600, etc.

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EVALUATION OF PREPARTUM ALTERNATIVE OILSEED MEAL SUPPLEMENTATION ON BEEF COW AND CALF PERFORMANCE

F.D. Loehr, C.L. Loehr, S.I. Paisley, T.L. Smith, and B.W. Hess
University of Wyoming, Laramie, WY

ABSTRACT: Pregnant cross-bred Angus cows (n=99, BW 654±12 kg, BCS 5.00±0.35) were assigned to nine pens to determine effects of three prepartum oilseed supplementation strategies on cow performance, and on that of their calves. Initial weight, age and BCS of cows were similar (*P*=0.78) across pens, with one of three treatments randomly assigned to each pen. Diets consisted of 11.6 $kg \bullet hd^{-1} \bullet d^{-1}$ mixed hay and one of three supplementation treatments formulated to meet energy, protein, and mineral requirements for cows in late gestation. Supplements consisted of either soybean meal and cracked corn (**CONTROL**), safflower and soybean meals (**SAFFLOWER**), or camelina meal (**CAMELINA**). After a 57-d feeding study, cows were weighed and body condition scored before calving. Within 24-h of calving, calf and cow data were recorded. Postpartum cows with live calves were managed as one group. Cow weight and condition at branding (d 49) and weaning (d 241) were also noted. Data were analyzed in a completely randomized design with pen as the experimental unit. All treatments achieved similar (*P*=0.48; 22 kg, 0.38 kg/d) weight gain and increased (*P*=0.86; +0.17 BCS) body condition during the feeding study. Calving ease and birth weight were similar (*P*=0.45; 1.07 score and 42 kg, respectively) across treatments. Cows on CONTROL diet tended (*P*=0.08) to have lower BCS than did animals on the SAFFLOWER treatment, with CAMELINA intermediate (4.93, 5.05, and 5.00, respectively). Body weight and BCS of cows at 49-d postpartum were similar (*P*=0.61; 673 kg, 4.64 BCS) across treatments. Cows bred back at similar rates (*P*=0.23, 90%), and death loss of calves was similar (*P*=0.87, 8%) across treatments. Adjusted 205-d weaning weights (*P*=0.80; 273 kg) were similar across treatments. Alternative oilseed meal supplementation prepartum yielded similar results to traditional corn and soybean supplementation on cow performance and fertility and on calf weights and death loss.

KEYWORDS: beef cattle, oilseed supplementation, performance.

Introduction

Recently, high plains livestock producers have faced high fuel prices, high corn prices, and high overall feed prices due to drought conditions. Corn and oilseed meal prices have also been largely impacted by the growing biodiesel and ethanol industry. As corn and soybeans are under heavy demand for human food and livestock feed use, alternative oilseeds are being analyzed both for potential oil production and livestock feed use. An ideal oilseed will yield high quantity and quality oil for biodiesel production, while also maintaining both protein and fat content for use by livestock. Two feasible options for oilseed production for biodiesel and feed as dryland crops in the Western high plains are safflower and camelina.

Whole safflower (*Carthamus tinctorius*) seeds can be high in linoleic or oleic unsaturated fatty acids. Linoleic acid is considered an essential omega-6 fatty acid, and is therefore desirable in livestock diets. Whole safflower seeds contain, on average, 17.5% CP, and 32% oil (Lardy, 2008). As an oilseed meal, safflower is of lower nutritive value than the industry standard of soybean meal.

Whole seed camelina (*Camelina sativa*) contains approximately 38% oil and 27% crude protein on a dry matter basis, and contains relatively high percentages of omega-3 fatty acids (Lardy, 2008). Feeding the omega-3 fatty acids may have positive impacts on omega-3 levels in meat for human consumption (Maddock et al., 2006), while also supporting the animal's own immune functions, as suggested by earlier supplementation studies conducted by Lake et al. (2006) and Lammoglia et al. (1999). Camelina is of particular interest in the high plains region of the U.S., as it has reduced input requirements, is more drought-tolerant, and may be suitable for marginal soils (Putnum et al., 1993). There is concern about feeding camelina to livestock, as it contains potentially harmful glucosinolate and tannin compounds, as well as erucic acid, all found in low levels in both the seeds and oilseed meal.

The objectives of this study are to evaluate feeding supplemental camelina meal to pregnant cows, and to determine whether alternative oilseed meals perform similarly to traditional soybean meal in cow performance and reproduction, calf performance and health, and calf immune response. We hypothesize that alternative oilseed meals and traditional soybean/corn supplements fed prepartum to beef cows will yield similar performance in cows and their progeny.

Materials and Methods

Animals

All procedures for the following experiment were approved by the University of Wyoming Animal Care and Use Committee. Ninety-nine pregnant multiparous cross-bred Angus cows from University herds were randomly assigned to nine pens to determine the effects of three pre-calving oilseed supplementation strategies on cow performance and on calf health and performance. Pens were similar (*P*=0.78) in cow body weight (654±12 kg), age (8 years, range of 5-12 years), and body condition score (5.00±0.35). Each pen was randomly assigned to one of three diet treatments.

Diets
Animals on all treatments were provided free-choice fortified trace mineral salt. Diets consisted of 11.6 kg·head^{-1}·d^{-1} mixed grass hay and one of three supplementation treatments (3% added fat, isocaloric, isonitrogenous) formulated to meet energy, protein, and mineral requirements for cows in late gestation. Supplements consisted of soybean meal and cracked corn (**CONTROL**), high linoleate safflower meal and soybean meal (**SAFFLOWER**), or camelina meal (**CAMELINA**).

Measurements & Analysis
At the start of the study period, cows were weighed, body condition scored, and had age verified by herd records. After a 57 d feeding study, and just before calving, cows were reweighed and body condition scored. Calving ease and body condition score were noted within 24 h of calving. Calf birth weight and vigor were also recorded within 24 h of birth, and blood samples taken from cows and calves were analyzed for T_3 and T_4 levels. Concentrations of T_3 and T_4 were determined using Coat-A-Count kits (DPC Diagnostic Products Inc., Los Angeles, CA) solid-phase $_{125}$I RIA. Cows with live calves were managed as a single group. Cow weight and condition were also measured at branding (d 49) and at weaning (d 241). Reproductive success was calculated as percent of cows verified pregnant by a licensed veterinarian as of d 225, after a CIDR synchronization protocol, first-heat AI service, and use of a clean-up bull. Adjusted 205-d weights were calculated from weaning weights (d 241). Calf death loss was determined as dead calves from live birth to weaning.

Immunology
Blood was drawn via venipuncture from cows (14 days before calving) and from calves (within 24 h of birth) for use in analysis of passive immunity transfer. Calves were challenged with a subcutaneous inoculation of egg ovalbumin B on d 93, with response measured on d 93, 100, 107, and 114. Response was measured using blood samples collected by venipuncture, and by calf rectal temperature. Immune response was measured using ELISAs.

Statistics
Data were analyzed in a completely randomized design using the GLM procedures of SAS (Version 9.1, SAS Inst., Inc., Cary, NC) with pen as the experimental unit. Least square means are reported, with means separated by least squares procedure when overall $P<0.05$. The SAS procedures CHISQ and FREQ were used to interpret reproductive distribution for individual cows, and death loss by individual.

Results and Discussion

Performance data for cows and calves are reported in Table 1. All treatments achieved similar weight gain ($P=0.48$; 22.0 kg; 0.38 kg/d) with similar corresponding increases in body condition ($P=0.86$; +0.17 BCS) during the feeding study, confirming that the treatment diets were isocaloric and provided adequate nutrition during late gestation. Calving ease and birth weight were similar ($P=0.45$; 1.07 score and 42.3 kg, respectively) across treatments. Birth weights were expected to be similar across treatments, as results from other studies that suggest that late gestation supplementation of fat does not affect birth weights of resulting calves, as reviewed by Funston (2004). Cows fed CONTROL diet tended ($P=0.08$) to have lower BCS at calf birth than did animals on the SAFFLOWER treatment, with CAMELINA intermediate (4.93, 5.05, and 5.00, respectively). Triiodothyronine (T_3) and thyroxine (T_4) were measured in both the cow and the calf to assess whether glucosinolates from camelina affected iodine metabolism, as high glucosinolates levels could result in a reduction of overall T_4 levels. The results of T_3 analysis in the cows suggested that animals fed SAFFLOWER tended to have greater T_3 than did those on the CONTROL diet, with CAMELINA intermediate ($P=0.06$, 69.2 vs. 61.6 and 66.3 ng/dL, respectively). Levels of T_4 were similar ($P>0.63$) across treatments for both cows and calves, as was T_3 in calves. Body weight and BCS of cows at 49 d postpartum were similar ($P=0.61$; 611 kg, 4.64 BCS) across treatments. Cows bred back at similar rates ($P=0.23$, 90%), and death loss of calves was similar ($P=0.87$, 8%) across treatments. The high death loss rate was partially attributed to an isolated soil fungus, which contributed to death by acute ruminal bloat. As expected from the similar birth weights and single-group treatment after birth, adjusted 205-d weaning weights ($P=0.80$; 273 kg) were similar across treatments.

Immunology
At the time of publication, no results are available for the passive transfer data. Results from the immune challenge are summarized in Figures 1 and 2. At each sampling time (0, 1, 2, 3 wk. post inoculation), there were no differences ($P>0.45$) in response between calves from dams on different treatments. Regardless of time after inoculation, there were no differences ($P>0.27$) in temperature between calves from dams on different treatments.

Figure 1. Impact of three prepartum beef cow supplementation strategies on immune response (absorbance) of their calves when inoculated with egg ovalbumin B at 93 days of age ($P>0.45$).

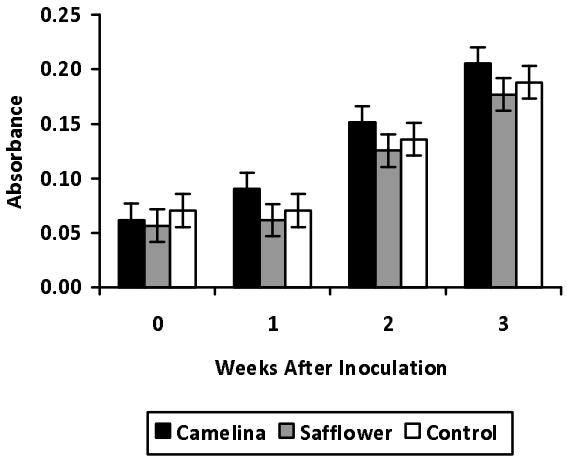
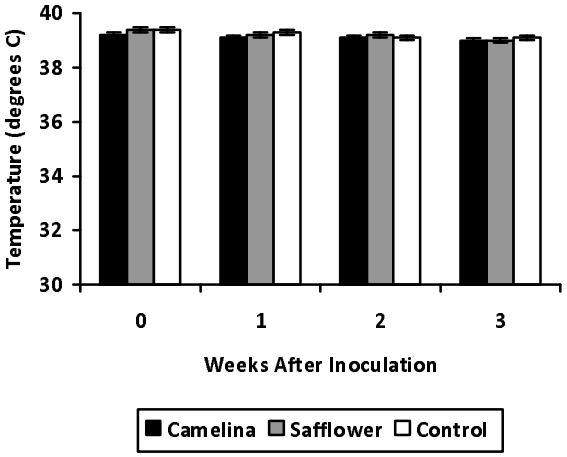


Figure 2. Impact of three prepartum beef cow supplementation strategies on immune response (temperature) of their calves when inoculated with egg ovalbumin B at 93 days of age (*P*>0.29).



Summary and Implications

Alternative oilseed meal supplementation yielded similar results to traditional corn and soybean meal supplementation on cow performance, subsequent reproductive ability, on calf birth weights, calf weaning weights, calf mortality, calf immune health, and on cow and calf metabolism. This data suggests that both safflower meal and camelina meal may be useful to Western high plains ranchers as an energy supplement fed prepartum to beef cows in late gestation. This data would also support the FDA's acceptance of camelina as a Generally Regarded as Safe feedstuff. The conclusion of this project will include the analysis of IgG passive transfer and the summary of calf feedlot performance and carcass characteristics after slaughter.

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Table 1. Impact of three oilseed supplementation strategies on performance of cows and their calves.

Item	CON[a]	SAFF[b]	CAM[c]	SE[d]	*P*-Value
Cows	33	33	33	-	-
Pens (9 hd/pen)	3	3	3	-	-
Initial BW[e], kg	654	654	654	11.7	1.00
Initial BCS (1-9)	5.00	4.99	5.01	0.06	0.99
Cow Weights & BCS					
End-trial BW[f], kg	678	679	674	11.8	0.95
End-trial BCS	5.15	5.19	5.17	0.06	0.89
Change in BW, kg	24.1	24.5	20.0	2.85	0.48
Change in BCS	0.15	0.20	0.17	0.06	0.86
Calving BCS	4.93[g]	5.05[h]	5.00[g, h]	0.04	0.08
Branding BW, kg	608	612	614	11.8	0.94
Branding BCS	4.60	4.65	4.68	0.06	0.61
At Calving					
Calving Ease	1.06	1.12	1.03	0.05	0.45
Calf Birth Wt., kg	42.1	41.7	43.0	1.2	0.74
Cow T_3, ng/dL	61.6	69.2	66.3	2.27	0.06
Cow T_4, ng/dL	14.1	14.9	14.5	0.15	0.73
Calf T_3, ng/dL	358	375	354	16	0.63
Calf T_4, ng/dL	14.1	14.9	14.5	0.7	0.70
Weaning					
Calf 205-adj., kg	271	274	274	4.5	0.80
% Calves lost	9.0	6.0	9.0	-	0.87
Cows Rebred	90	83	97	-	0.23

[a] Control diet consisting of alfalfa/grass hay, and supplement with corn and soybean meal, calculated to meet requirement of cows in late gestation.

[b] Safflower-based diet consisting of alfalfa/grass hay, safflower and soybean meals to provide for requirement of cows in late gestation.

[c] Camelina diet consisting of alfalfa/grass hay, and camelina meal supplement to provide for requirement of cows in late gestation.

[d] Data analyzed as a completely randomized design using GLM of SAS. Standard error is reported.

[e] *P*-values are considered significant when $P < 0.05$.

[f] Initial and final weights determined by averaging two consecutive day weights.

[g,h] Calving BCS $P = 0.08$, trend is noted for means with different superscripts.

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BEEF SYSTEM METHODS IMPACT BACKGROUNDING AND FINISHING NET RETURNS

D.G. Landblom[1], K.C. Olson[2], P. Johnson[2], R. Gates[2], M.K. Beutler[2], M. Hubert[2], S.W. Fausti[3], G.A. Perry[3], R.R. Salverson[4], I. Rush[5], S. Furman[5], J. Vasconcelos[5]

[1]Dickinson Research Extension Center, North Dakota State University
[2]West River Agricultural Center, South Dakota State University
[3]Departments of Animal Science and Economics, South Dakota State University
[4]Cooperative Extension Service, South Dakota State University
[5]Panhandle Research Extension Center, University of Nebraska

Abstract: Cow-calf pairs, grazing native range, from the NDSU-Dickinson RE Center and the SDSU-West River Ag Center (n = 159) were used to evaluate weaning date and backgrounding method. Treatments were: 1) Normal Wean (Jun-Nov) - feedlot direct (NW-FLT), 2) Early Wean (Aug) – feedlot direct (EW-FLT), 3) Early Wean (Aug) - grazed dryland unharvested corn (Aug-Nov) - feedlot (EW-CN), and 4) Normal wean (Nov) - grazed dryland unharvested corn (Nov-Dec) - feedlot (NW-CN). Feedlot arrival date for finishing at the UNL-Panhandle RE Center feedlot, Scottsbluff, NE was staggered. Harvest end point was based on ultrasound BF depth. Mean differences were determined using the SAS MIXED procedure. For backgrounding, EW-CN and EW-FLT steer growth was similar and more rapid [(Gain: (P = 0.043) and ADG: (P = 0.004)] than NW-FLT and NW-CN. The EW-CN system COG of $1.05/kg was lowest when compared to $1.31, $3.77, and $1.37/kg for the NW-FLT, NW-CN, and EW-FLT, respectively. Stockpiling corn resulted in excessive crop shrink (P = 0.013) reducing days of grazing by 70%. Backgrounding net returns/steer were $87.50, -$33.38, $104.58, and $69.56 for the NW-FLT, NW-CN, EW-CN and EW-FLT, respectively. For finishing, EW-FLT steers grew slower (P = 0.0011), consumed less DM/d (P = 0.0001), were more efficient (P = 0.008), and COG was lower (P = 0.0002). Carcass closeout values for HCW, FD, dressing %, and YG did not differ; however, EW-FLT steer carcasses had smaller REA (P = 0.053), greater marbling score (P = 0.0005), and numerically greater % Choice quality grade (P = 0.11). EW-FLT steers placed directly in the feedlot at weaning were associated with lower placement cost, more DOF (P = 0.0001), and higher feed and yardage costs. Net return to finishing of $39.62 per head for the EW-FLT was greater, when compared to $3.11, -$84.06, and $0.16 for the NW-FLT, NW-CN, and EW-CN, respectively. Experimental results suggest that greatest beef systems net return will be obtained when EW steers graze dryland unharvested corn and are sold at the end of backgrounding; however, when held through final harvest, early weaning and direct feedlot placement were associated with greatest net return.
Key Words: Beef Systems, Early Weaning, Corn Grazing

Introduction

Previous research has evaluated forage utilization by early (August - EW) vs normal (November - NW) weaned beef cows and the effect of weaning date on cow and calf performance. These studies show that weaning calves early has a positive impact on growth and efficiency during the backgrounding phase, improves cow body condition score, reduces range forage utilization, and shortens the lifetime feeding period of steers held for retained ownership (Landblom et al., 2006). Economic analysis of retained ownership concluded that early weaning improved feedlot production efficiency by reducing daily and per carcass revenue relative to normal weaning (Fausti et al., 2007). And subsequently, Landblom et al. (2008) documented that significantly altering

weaning date can have a positive impact on business profitability in the beef cattle enterprise. The objective of this study was to evaluate the effect of weaning date (August vs November) and backgrounding method on backgrounding and finishing net returns.

Materials and Methods

Spring calving cows (Mar-Apr) originating at the South Dakota State University Antelope Station (ANT), Buffalo, SD, and the North Dakota State University Dickinson Research Extension Center (DREC), Manning, ND were used in a 2 x 2 factorial arrangement comparing weaning date (August vs November) and backgrounding method (feedlot vs grazing dryland unharvested corn). Pen or pasture served as the experimental unit and backgrounding, finishing, and carcass data were analyzed using the SAS MIXED procedure. The protocols used in this study were approved by the North Dakota State University Animal Care and Use Committee.

Steer calves in the EW system were weaned on August 15 and calves in the NW system were weaned the first week of November. At each weaning date, steers from each research facility were randomly assigned to either feedlot or corn grazing backgrounding treatments. Corn grazing steers were held in drylot and fed hay for two weeks before being put into replicated dryland unharvested corn fields. Early weaned steers began grazing unharvested corn on August 25th and the NW steers began grazing corn on November 21st. For the feedlot treatment, EW and NW steers were shipped by commercial truck to the University of Nebraska Panhandle Research Extension Center feedlot, Scottsbluff, Nebraska where they were finished and harvested at a commercial Abattoir. Steer weight and backfat depth of 12.7 mm were used to determine final harvest endpoint. Measurement for backfat depth was conducted 30 – 45 days before final harvest using a SonoVet ultrasound machine and 3.5 MHz probe. Final harvest date was determined by calculating the required number of DOF to attain 12.7 mm BF.

Systems measurements were: corn forage nutrient change, corn forage utilization, backgrounding performance type and economics, treatment effect on animal health, corn grazing grain equivalent value, finishing performance and economics, and carcass closeout values.

Steers in the systems investigation were vaccinated before spring turnout on native pasture and then were vaccinated 3-4 weeks before each weaning date, and again at weaning with modified live IBR, BVD types I and II, PI_3, BRSV + Mannhiema haemolytica, and an inactivated 7-way Clostridial vaccine + H. somunus. In addition, the calves were poured with a parasiticide. After weaning, the calves were observed closely for the onset of health problems and were treated according to the attending veterinarian's recommendation. The following information is being recorded: body temperature, number of pulls, product used for treatment and cost, percent death loss, and system cost due to death loss.

Results and Discussion

Systems Backgrounding - Considering the results of Fausti et al. (2007) in the previous study, the present investigation was conducted to compare calf growing methods for EW and NW calves after weaning that compared feedlot backgrounding with grazing unharvested dryland corn before finishing based on a high quality grid. Standing peak dryland corn forage nutrient quality was determined mid-September and tracked through to mid-January. Corn forage CP declined from Sep to Nov (9.16 to 8.66) and IVDMD declined from 75.2% to 57.0%.

Peak DM corn production for the EW steers averaged 2.0 MTon/acre and peak DM corn production for the NW group was 1.75 MTon/acre. Early weaned steers utilized an average 1.46 MTon/acre over the 70 day grazing period and NW steers utilized 0.37 MTon/acre. Field loss in stockpiled corn set aside for grazing after normal weaning was excessive averaging 0.82 MTon/acre. Compared to the EW treatment, the large field loss reduced available days of grazing by 70%.

Comparative systems backgrounding performance is shown in Table 1. Steer weight at EW did not differ (P=0.44), but gain among the NW-CN steers was reduced significantly (P=0.043) due to field crop shrink. Average

daily gain for EW and NW steers was similar and greater (p=0.004) than the control steers despite significant crop shrinkage. System backgrounding economics are shown in Table 2 where gain value, input costs, net returns, and cost/kg of gain are summarized. The backgrounding cost/kg of gain was $$1.31, $3.77, $1.05, and $1.37 for the NW-FLT, NW-CN, EW-CN, and EW-FLT, respectively. Net return/steer among the steers in EW-CN system was 33.5% greater than the EW-FLT system and 16.3% greater than the NW-FLT system. Stockpiling corn for grazing after normal weaning was not successful resulting in a net loss/steer of -$33.38. The stocking rate for early weaned calves that graze unharvested dryland corn was calculated to be 0.1012 hectare/weaned calf/month and the stocking rate for stockpiled corn reserved for normal weaned calves in the study was determined to be 0.324 hectare/weaned calf/month.

The effect of alternative weaning date and corn grazing on finishing performance is shown in Table 3. Early weaning and corn grazing backgrounding resulted in staggered feedlot start weight (P = 0001), and a large variation in the number of days on feed (P = 0.0001); however, harvest age (P = 0.27) and 4% shrunk harvest weight (P = .409) did not differ. For gain and FE, EW-FLT steers gained at the slowest rate (P = 0.001), were more efficient (P = 0.008), and feed and yardage cost/kg of gain (P = 0.0002) were lower. By contrast, EW-CN steers that were the most profitable at the end of corn grazing backgrounding were less efficient (P = 0.008) and feed and yardage cost/kg of gain was higher (P = 0.0002). The NW-CN steers that grazed stockpiled dryland corn were the least efficient (P = 0.008) and had the highest feed and yardage cost/kg of gain (P = 0.0002).

Carcass closeout values for HCW (P = 0.78), dressing percent (P = 0.51), fat depth (P = 0.243), and yield grade (P = 0.23) did not differ. Corn grazing steers had significantly larger ribeye area (p = 0.053). Days on feed, which varied due to management system, directly affected marbling score (P = <0.0001) and the number of carcasses that graded USDA Choice or better (P = 0.10).

The combined effect of calf placement cost, ingredient cost, treatment cost, freight, and interest factors affected finishing net return. Calf placement cost had the most influence on net return. Closeout net returns were $3.11, -$84.06, $0.16, and $39.62/head for the NW-FLT, NW-CN, EW-CN, and EW-FLT, respectively.

Implications

Results suggest that greatest beef systems net return will be obtained when EW steers graze dryland unharvested corn and are sold at the end of backgrounding; however, when held until final harvest, early weaning and direct feedlot placement were associated with greatest net return.

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Table 1. Systems Backgrounding Performance

	NW- Ctrl Pasture/ Feedlot	*NW – Corn Grazing*	*EW – Corn Grazing*	*EW – Feedlot*	*SE*	*P-Value*
Weaning Date	Nov 7	Nov 7	Aug 15	Aug 15		
No. Steers	54	24	24	57		
System Days	84	21	70	86		
System Weaning Wt., kg[a]	197.9	289.8	212.3	183.7	10.02	0.44
System End Wt., kg	272.2	314.3	300.3	277.1	15.05	0.15
Gain, kg	74.5[ab]	24.5[b]	88.0[a]	93.4[a]		0.043
ADG, kg	0.887[b]	1.16[a]	1.26[a]	1.09[a]	0.057	0.004

[a]Weaned steers were held in drylot for 13 days before placement in the corn fields to get over weaning.

Table 2. Alternative Beef System Unharvested Corn, Pasture, and Feedlot Economics

	NW- Ctrl Pasture/ Feedlot	*NW – Corn Grazing*	*EW – Corn Grazing*	*EW – Feedlot*
No. Steers	54	24	24	57
Gain Value [a,b,c,d]	$9,979	$1,413	$4,724	$10,980
Input Cost:				
Pasture (Rent @$14.00/ac)[e]	$5,254			
Corn ($164/ac)		$2,214	$2,214	
Feedlot				$7,302
Backgrounding Net Return	$4,725	-$801	$2,510	$3,678
Backgrounding Net Return/Head	$87.50	-$33.38	$104.58	$69.56
Cost/kg Gain	$1.31	$3.77	$1.05	$1.37

[a]NW Control Gain Value (8,910lb@$112/cwt)
[b]NW Corn Grazing Gain Value (4,334lb@$109/cwt)
[c]EW Gain Value (1,296lb@$109/cwt)
[d]Gain Value (9,804lb@$112/cwt)
[e]Pasture Rent Calculation: 2.78 months, 2.5 AUM; = 6.95 Ac/AUM @ $14/Ac; = $97.30 x54 = $5,254.20

Table 3. Effect of Alternative Weaning Date and Corn Grazing on Steer Finishing Performance

	NW- Ctrl Pasture/ Feedlot	*NW – Corn Grazing*	*EW – Corn Grazing*	*EW – Feedlot*	*SE*	*P-Value*
Start Wt., kg	272.2[c]	339.2[b]	313.1[d]	183.6[a]	37.2	<0.0001
4% Shrunk End Wt., kg[a]	538.4	555.2	566.9	545.7	10.44	0.409
Days on Feed	192[d]	141.5[b]	165.7[c]	280.8[a]	3.44	<0.0001
Kill Age, Days	408.1	415.1	404.6	412.1	3.17	0.270
ADG, kg	1.39[b]	1.53[c]	1.53[c]	1.29[a]	0.025	0.0011
DM Fd/Head/Day, kg	9.12[b]	11.1[d]	10.2[c]	8.07[a]	0.23	<0.0001
DM Feed:Gain, kg	6.56[b]	7.26[c]	6.64[b]	6.26[a]	0.072	0.008
Fd & Yard Cost/Day, $	$2.096[b]	$2.723[d]	$2.383[c]	$1.715[a]	0.053	<0.0001
Fd & Yard Cost/kg of Gain, $	$1.51[b]	$1.78[c]	$1.56[b]	$1.33[a]	0.016	0.0002

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DETERMINING THE VIABILITY OF COMPOSTING ON-FARM FEEDSTUFFS AND ANIMAL WASTE IN NORTHERN MONTANA

J.M. Dafoe and D.L. Boss
Montana State University, Northern Agricultural Research Center, Havre, MT 59501

ABSTRACT: The objective of this demonstration project was to illustrate the viability of composting in northern Montana during winter months. The compost consisted of 163.3 t of dried cattle manure and bedding material from a permitted feedlot, 38.1 t of wheat straw, and 38.1 t of year-old spoiled corn silage. Wheat straw and corn silage were produced at Northern Agricultural Research Center. Two windrows 66.8 m by 3.7 m and 76.8 m by 3.7 m of the manure blend were constructed on a flat surface of clay loam soil. Moisture content was analyzed to be 12.5% at the beginning of the project. The compost was turned with an elevating face Vermeer compost turner twice weekly, weather permitting. A 90 cm data logger was placed into the windrows 3 times weekly to record internal temperature and oxygen levels. Moisture of each windrow was measured with a hay probe 3 times weekly. After initial readings, water was added to the windrows bringing the moisture content to approximately 50%. Ambient temperature readings were recorded daily at 0800 h. Maximum and minimum mean daily ambient temperatures during the study were 6.9°C and -31.1°C, respectively. Windrow 1 and W2 reached a high temperature of 60.6°C 7 d after irrigation and 63.6°C 9 d after irrigation respectively. Mean core temperatures in W1 and W2 exceeded 40°C 120 h and 24 h after irrigation, respectively. Core temperatures remained above 40°C for 26 d in W1 and 30 d in W2. During this time, core temperatures were continuously above 55°C for 19 d in W1 and 21 d in W2. Even though the minimum ambient temperature reached -31.1°C, there was no lasting negative effect. Once 40°C was reached in W1, the compost matured at the same rate as W2. During the coldest period of the experiment core temperatures remained above 35°C and 47°C in W1 and W2, respectively. It was determined that composting is a viable option in northern Montana during the winter months and further research is planned.

Key words: Compost, Animal Waste, Beef

Introduction

Montana has many small and medium animal feeding operations, including those that have seasonal or temporary confinement. Composting methods for manure are not validated in the literature for Montana. Documenting successful composting and evaluating markets for the finished product will provide a Montana specific case study, with data, for other potential producers to examine before adding this sustainable practice to their operations. In order for Extension, the Land Grant University and other partners to move forward with educational programs and recommendations, this validation needs to occur. This practice can also provide a local and potentially lower cost input for other businesses. Compost can serve as an excellent soil amendment and nutrient source. This project will seek to validate composting recommendations for manure in Montana's cold semi-arid environment. Exporting manure nutrients in the form of compost will provide for better nutrient balance on the operations and enable other users such as homeowners and gardeners, commercial nurseries and landscapers, organic producers and others to utilize this organic based fertilizer and soil amendment. Manure and manure based products, such as compost, are two alternatives to traditional energy intensive products (Bass et al., 2008).

Materials & Methods

The compost consisted of 163.3 t of dried cattle manure and bedding material from a permitted feedlot, 38.1 t of wheat straw, and 38.1 t of year-old spoiled corn silage to bring the carbon:nitrogen (C:N) ratio to a calculated value of approximately 30:1. The C:N ratio was within the recommended optimum range for US composting guidelines (Table1). Wheat straw and corn silage were produced at Northern Agricultural Research Center. Two windrows (W1 and W2) 66.8 m by 3.7 m and 76.8 m by 3.7 m of the manure blend were constructed on a flat surface of clay loam soil. Moisture content was analyzed to be 12.5% at the beginning of the project shortly after the compost materials were blended and the windrows were constructed. The compost was turned with an elevating face compost turner (CT-670, Vermeer, Pella, IA) twice weekly, weather permitting. A 90 cm data logger (Windrow Manager, Green Mountain Technologies, Bainbridge Island, WA) was placed into the windrows 3 times weekly to record internal temperature and oxygen levels of each windrow at a depth of 45 and 89 cm. Moisture of each windrow was measured with a hay probe (DHT-1, Farmex Electronics, Streetsboro, OH) 3 times weekly. After initial readings, water was added to the windrows as they were being turned, bringing the moisture content for each windrow to approximately 50% (Trautmann et al, 1996). Water addition took place on December 3 and 5, 2008. Ambient temperature readings were recorded daily at 0800 h (National Oceanic and Atmospheric Administration, National Weather Service Cooperative Observer Site: Fort Assiniboine; Site ID: ASNM8; Site Number: 24- 110-03: Lat/Lon: 48.29.54, 109.47.50: Elevation: 2613 ft.).

Results and Discussion

Maximum and minimum mean daily ambient temperatures for the 47 d trial were 6.9°C and -31.1°C, respectively. From the beginning of the trial to when the windrows were irrigated (d 16 and 14, respectively) no composting or aerobic activity was occurring evidenced by the low windrow temperatures (mean temp W1 and W2; Figure 1) and high oxygen levels (Figure 2). Oxygen levels in the windrows were >16% and >6% before irrigation in W1 and W2, respectively. After irrigation aerobic bacteria began immediately to start digesting the nitrogen and carbon sources within the windrows.

Mean core temperatures in W1 and W2 were >40°C 120 h and 24 h after irrigation, respectively (Table 1). Composting occurs most rapidly when temperature are >40°C (Trautmann et al, 1996). Oxygen levels returned to 20% immediately after aerating (turning) the windrows, however within 24 h of turning, oxygen levels were < 2% indicating a very rapid aerobic digestion of feedstocks and available oxygen. Windrow 1 and W2 reached a high temperature of 60.6°C 7 d after irrigation and 63.6°C 9 d after irrigation, respectively. Oxygen levels below 5% result in anaerobic conditions within the windrows (Trautmann et al, 1996; Figure 2), hence the requirement for such frequent turning of the windrows in this trial. The wheat straw and year old corn silage in this composting system broke down quickly and added little to the overall bulk density. To combat the low oxygen levels in the center of the windrows, our procedure resulted in turning quite frequently. In future compost trials, adding different carbon sources including whole corn stalks or wood chips for bulking agents may promote passive aeration and oxygen availability.

Composting systems can achieve a significant reduction of pathogens when the compost is maintained at minimum operating conditions of 40°C for five days, with temperatures exceeding 55°C for at least four hours of this period (Trautmann et al., 1996). Core temperatures within this trial remained above 40°C for 26 d in W1 and 30 d in W2 (Figure 1). Even though the minimum ambient temperature reached -31.1°C, there was no lasting negative effect as the compost was able to generate appropriate temperatures after each aeration. Microbial activity was sufficient for composting as evidenced by the independent temperature trends of windrow temperature and ambient temperature. During the coldest period of the experiment (d 25-33°C), ambient temperature was <-17°C for 6 d; and core temperatures remained above 35°C and 47°C in W1 and W2, respectively. During the trial there was 4.22 cm measurable precipitation in the form of snow, with 1.42 cm falling on d 2. Precipitation events had no apparent effect on temperature, oxygen, or moisture content of the compost. Possibly because the precipitation came as snow and the ambient temperatures did not allow it to add to the moisture content of the windrow. Core temperatures were continuously above 55°C for 19 d in W1 and 21 d in W2. For windrow composting methods, maintaining 55°C for 15d is sufficient to destroy weed seed viability (Trautmann et al, 1996; Wilen, 1997). Once the temperature of the windrows maintain 55°C, the environment in compost windrows have sufficient moisture and temperature to break dormancy of hard seeds followed by thermal kill of seedlings (Egley, G.H., 1990). Once 40°C was reached in W1 the compost matured at the same rate as W2 as indicated by temperature and oxygen readings. Compost was deemed to be mature when it conformed to US Composting Council guidelines including: C:N ratio, pH, organic matter, and moisture content (Alexander, 2003).

Implications

Composting can occur at any time with on farm nitrogen and carbon sources. In this trial, in the semi-arid region of Northern Montana, the most important factor was to reach the appropriate moisture content (>35%) after blending the carbon and nitrogen sources to initiate the aerobic composting process. Perhaps less turning (aerating) would be required with larger bulker carbon sources rather than our choices of wheat straw and corn silage. It was determined that composting is a viable option in northern Montana during the winter months and further research is planned.

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Table 1. Beef cattle manure with bedding and final compost sample nutrient analyses of two compost windrows (W1 and W2). Initial blends of compost feedstocks included 163.3 t of dried cattle manure and bedding material from a permitted feedlot, 38.1 t of wheat straw, and 38.1 t of year-old spoiled corn silage to bring the carbon:nitrogen (C:N) ratio to a calculated value of approximately 30:1

Nutrient	Manure and Bedding	Wheat Straw	Corn Silage	Final Compost
Carbon, %	28.0	14.2	51.3	7.9
Nitrogen, %	2.2	0.4	2.7	1.3
Carbon:Nitrogen	13:1	35.5:1	19:1	9.1:1
pH	8.5	-	-	8.3

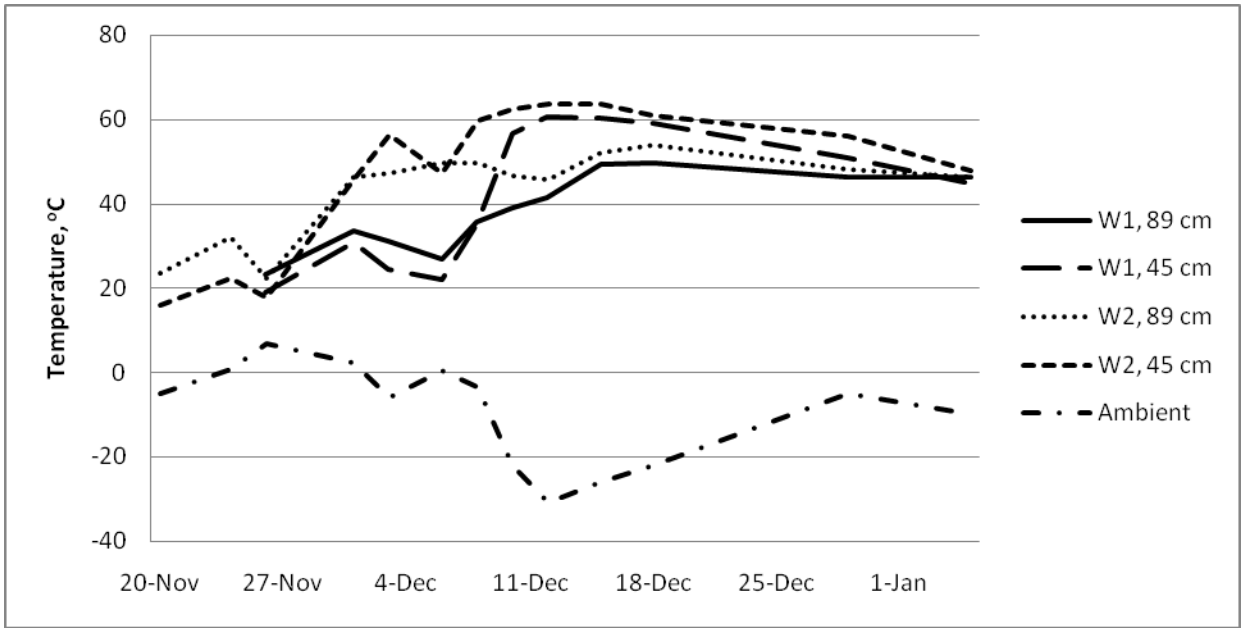

Figure 1. Mean core temperatures of two compost windrows (W1 and W2) composed of 163.3 t of dried cattle manure and bedding material from a permitted feedlot, 38.1 t of wheat straw, and 38.1 t of year-old spoiled corn silage to bring the carbon:nitrogen (C:N) ratio to a calculated value of approximately 30:1; and associated mean ambient temperatures near Havre, Montana. After initial readings, water was added to the windrows as they were being aerated (turned) bringing the moisture content for each windrow to approximately 50%. Water addition took place on December 3 and 5, 2008.

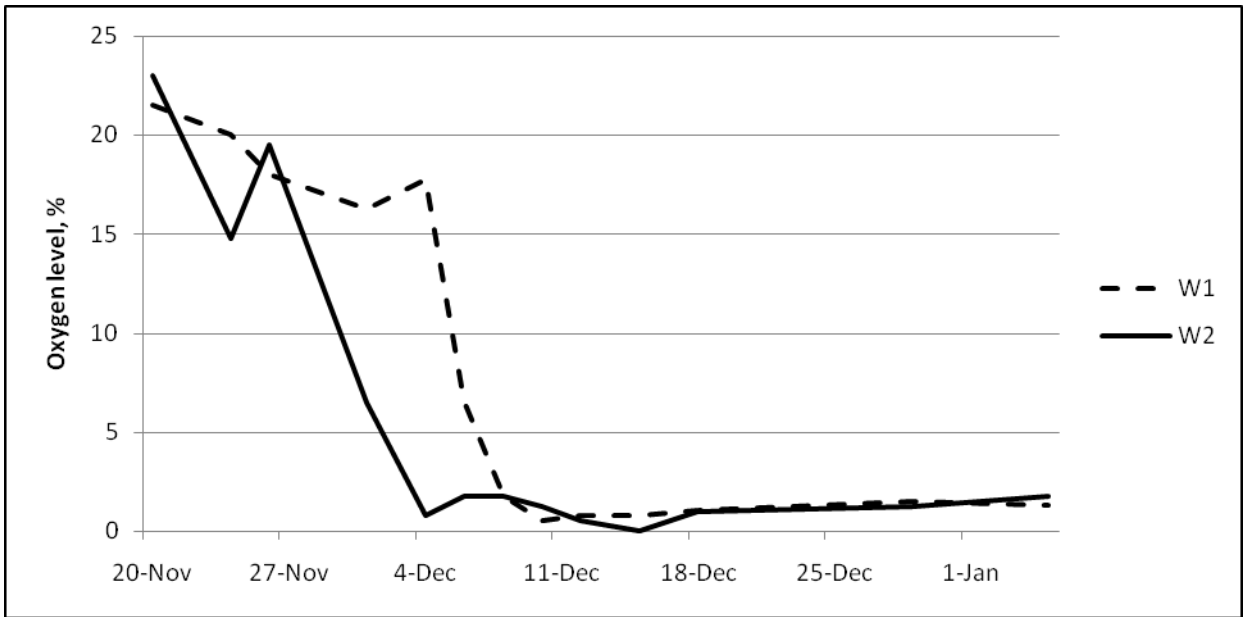

Figure 2. Oxygen levels of two compost windrows (W1 and W2) composed of 163.3 t of dried cattle manure and bedding material from a permitted feedlot, 38.1 t of wheat straw, and 38.1 t of year-old spoiled corn silage to bring the carbon:nitrogen (C:N) ratio to a calculated value of approximately 30:1. After initial readings, water was added to the windrows as they were being aerated (turned) bringing the moisture content for each windrow to approximately 50%. Water addition took place on December 3 and 5, 2008.

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BREEDING PERFORMANCE OF SUFFOLK EWES ADMINISTERED SUBACUTE LEVELS OF DIETARY NITRATE.

R. R. Cockrum, K. J. Austin, K. L. Kessler, S. M. Rustemeyer, W. J. Murdoch, K. M. Cammack
Department of Animals Science, University of Wyoming, Laramie, WY

ABSTRACT: Accumulation of nitrite (NO_2^-) in ruminant animals due to high dietary nitrate (NO_3^-) intake leads to methemoglobin formation, resulting in toxicity. The effects of elevated dietary NO_3^- intake during gestation have been established; however, the effects of high dietary NO_3^- intake on female fertility are relatively unknown. The objective of this study was to determine the effects of subacute dietary NO_3^- administered immediately prior to breeding on ewe fertility. Purebred Suffolk ewes (n = 25) were synchronized by CIDR and randomly allotted to one of two treatment groups prior to breeding: 0 mg/kg BW KNO_3 (control; n = 10) or 175 mg/kg BW KNO_3 (NO_3^- treated; n = 15) by drench daily for two estrus cycles (26 d). Receptivity was confirmed using a vasectomized ram prior to treatment and during first estrous. Upon second estrous, ewes mated with two intact rams. Ewes were weighed weekly prior to conception, and blood was collected daily by jugular 3 d prior to treatment, throughout treatment (26 d), mating (5 d), after treatment until implantation (20 d), and once monthly until parturition. Data were analyzed using GLM and MIXED procedures in SAS assuming an alpha level of 0.05. Plasma samples were analyzed for progesterone, NO_3^-, NO_2^-, urea N, and ammonia. Corpora lutea were counted by laparoscopy and ultrasounds were conducted on d 70 to confirm pregnancy and count fetuses. Initial BW did not differ ($P = 0.56$) between control and NO_3^- treated ewes. There was an increased ($P = 0.001$) number of corpora lutea in treated ewes compared to controls; however, the number of fetuses present did not differ ($P = 0.14$) between treatment groups. Progesterone, ammonia and NO_2^- levels did not differ ($P \geq 0.50$) between treatment groups. Plasma NO_3^- ($P < 0.0001$) and urea N ($P = 0.08$) were higher in treated ewes. Results indicate that administration of subacute dietary NO_3^- prior to breeding does not affect ewe fertility.

Key words: Infertility, nitrate, reproduction, sheep

Introduction

Nitrate is converted to NO_2^- in the rumen and then further reduced to NH_4. Toxicosis results when NO_2^- levels accumulate as a result of increased dietary NO_3^-. Increased NO_2^- levels result in the conversion of hemoglobin to methemoglobin, whereby the ferrous ion of hemoglobin is transformed to the ferric form, reducing the ability of blood to carry oxygen to the body. Subacute NO_3^- toxicity affects early reproduction causing: infertility, conception difficulties, embryonic loss, fetal resorption, and early abortion when the embryo is most vulnerable (Page et al., 1990). During 1954, over 400 abortions in cattle were linked to grazing unmaintained fields and pastures that contained wild plants high in NO_3^- in Wisconsin (Sund and Wright, 1956). Currently, there is much speculation regarding the mechanism and effects of subacute levels of dietary NO_3^- on reproduction. According to Muhrer et al. (1956), 1% of KNO_3 in the diet can cause abortions or decrease milk production in cows; however, research has yet to reproduce the abortifacient effects of NO_3^- when administered from implantation to parturition. Eppson et al. (1960) noted that a diet containing 1.5% KNO_3 resulted in toxicosis, but had no harmful effect on pregnancies or growth of lambs. In cattle, NO_3^- has been shown to cross the placental barrier and diffuse into fetal amniotic fluid (El Bahri et al., 1997). Page et al. (1990) suggested that low levels of NO_3^- administered over an 8 wk period are capable of decreasing serum progesterone (P_4) concentrations during the luteal phase and excess dietary NO_3^- suppresses luteal production of P_4. The objective of this study was to 1) determine the effects of subacute dietary nitrate on ewe fertility when administered just prior to the breeding period, and 2) analyze circulating P_4 levels throughout and after treatment. We hypothesize that subacute dietary NO_3^- administered to ewes prior to breeding will decrease fertility.

Materials and Methods

Animal protocol. All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee. Purebred Suffolk ewes were randomly allotted to one of two treatment groups based on initial weight (82.55 ± 2.51 kg) prior to breeding. Diets consisted of alfalfa hay and a corn based supplement fed according to NRC requirements for non-pregnant ewes (NRC, 2007). Potassium nitrate (KNO_3) was mixed in a 1.5 molar solution prior to treatment. Treatment groups consisted of either control (0 mg/kg KNO_3 tap water only; n = 10) or treated (175 mg/kg KNO_3 mixed with tap water; n = 15) administered by drench and adjusted for weekly BW. An adjustment period of 3 d was given during which ewes were drenched only with the control treatment. Ewe estrus was synchronized by CIDR. Following removal of CIDR, ewes began respective treatment the following day for two estrous cycles (26 d). A vasectomized ram was used to check receptivity at the beginning of treatment and during first estrous. Upon second estrous ewes were removed from treatment and randomly divided into one of two

contemporary groups where two intact rams per group (n = 4) were used for mating. Once a ram marked a ewe, she was transferred to the next ram for cleanup and heat confirmation. After the mating period (7 d), ewes were allowed to gestate until parturition. Ewes were weighed weekly (prior and during treatment) and blood collected from the jugular vein daily prior to treatment (3 d), during treatment (26 d), during mating (7 d), until implantation (21 d), and once monthly until parturition. Laparoscopies were conducted 25 - 30 d post conception to count corpora lutea (CLs) and ultrasounds were completed on d 70 of gestation for pregnancy confirmation.

Plasma analyses. Blood was collected daily via the jugular vein 3 hours after treatment into tubes containing K^+ EDTA (Tyco Healthcare Group LP, Mansfield, MA) to prevent clotting. This time was chosen for blood collection according to when NO_2^- levels peak, which aids in the estimation of NO_3^- metabolic variation among treated ewes. All samples were immediately mixed and put on ice for 30 min. Samples were then centrifuged for 20 min at 1520 × G at 2° C after which plasma was obtained and stored at -20° C. Progesterone concentrations were analyzed using an RIA Coat-a-Count kit (Diagnostic Products Corp., Los Angeles, CA) on a Cobra II autogamma counter (Packard, Downers Grove, IL). All results were compared against internal controls of non-specific bound and standard samples. Progesterone was measured for the 57 consecutive days of blood collection and the monthly collections until parturition. Plasma urea nitrogen (PUN), NO_3^-, and NH_4 were analyzed during first week of treatment (d -1 to 5) and once weekly (d 11, 18, 25, and 32). Plasma NO_2^- levels were analyzed for the same days with the exception of d 32. Plasma urea nitrogen levels were confirmed using a QuantiChrom™ Urea Assay Kit (DIUR-500; BioAssay Systems, Hayward, CA) and measured on a Beckman Coulter DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA). The urea assay kit utilizes a chromogenic reagent that forms a colored complex specifically with urea (Jung et al., 1975). Nitrate and NO_2^- levels in plasma samples were analyzed using a Standard Range Lab Nitrate Test Kit (L-NTK; NECi, Lake Linden, MI) and measured on a Beckman Coulter DU 640 spectrophotometer (Beckman Coulter, Fullerton, CA). In this assay, NO_3^- was measured by the reduction of NO_3^- to NO_2^- using NO_3^- reductase as an electron donor, NADH (Campbell et al., 2006). Nitrite levels in plasma were determined with the omission of the NO_3^- reductase and NADH reagents from the assay. Plasma NH_4 levels were analyzed within 2 weeks of blood collection due to the instability of the compound in the plasma. In this assay NH_4 reacts with α – ketoglutarate and reduces nicotinamide adenine dinucleotide phosphate (NADPH) to form L-glutamate and NADP, which is catalyzed by glutamate dehydrogenase (Mondzac et al., 1965).

Laparoscopy. Laparoscopies were performed on all ewes on d 25 – 30 of gestation. Ewes were removed from food and water prior to surgery (72 hr and 48 hr, respectively). Ewes were anesthetized by intravenous injection of Xylazine HCl (0.3 mg/kg BW; Tranquived, VEDCO, St. Joseph, MO) and Ketamine HCl (3 mg/kg BW; Ketaset, Animal Health, Fort Dodge, IA). Incision areas on the abdomen were sheared and cleaned with chlorhexidine gluconate (1 oz; Fisher Scientific, Hampton, NH) for disinfection. Operative area was then sterilized with Betadine (Fisher Scientific). Two abdominal incisions (length of scalpel) were made, one for the scope and the second for manipulation of ovaries. Ovaries were examined for presence of CLs and any abnormalities. Following the procedure, ewes were administered an antibacterial injection of Penicillin to guard against infection.

Ultrasound. Fetuses were counted and pregnancy confirmed on d 70 gestation using ultrasound.

Statistical analyses. Change in BW, CLs, and fetal numbers were analyzed for effects of treatment (control vs. treated) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Plasma parameters, including P_4, NO_3^-, NO_2^-, urea N, and NH_4, were analyzed for the random effects of treatment (control vs. treated) and treatment × day using repeated measures in PROC MIXED, assuming an alpha level of 0.05.

Results

Body weights did not differ ($P = 0.56$; Figure 1) between control and NO_3^- treated ewes; however, control ewes were 0.94 kg heavier than treated ewes after 6 weeks. Plasma urea N tended to differ ($P = 0.08$; Figure 2) between treatment groups. In addition, NO_3^- levels were higher ($P < 0.0001$; Figure 3) in NO_3^- treated ewes than controls during the treatment period. Plasma NO_2^- and NH_4 levels did not differ ($P \geq 0.50$; Figure 4 and 5, respectively) between control and NO_3^- treated ewes. Circulating P_4 levels also were not different ($P = 0.57$; Figure 6) between control and NO_3^- treated ewes. There were more ($P = 0.001$; Table 1) CLs in treated ewes than controls; however, the number of fetuses present did not differ ($P = 0.14$) between treatment groups. Live birth and stillbirth numbers did not differ ($P > 0.33$) between control and NO_3^- treated ewes; however, gestation length tended ($P = 0.08$) to be shorter in NO_3^- treated ewes.

Table 1. Mean corpora lutea and fetal parameters of control and NO_3^- treated ewes.

Item	Control[1]	NO_3^- treated[2]	*P* - value
Corpora lutea	0.89 ± 0.21^a	1.92 ± 0.17^b	0.001
Fetal count	1.0 ± 0.20^a	1.4 ± 0.17^a	0.14
Live births	1.5 ± 0.35^a	1.38 ± 0.28^a	0.80
Stillbirths	0.25 ± 0.29^a	0.62 ± 0.23^a	0.33
Gestation length	146.0 ± 0.53^a	144.8 ± 0.41^a	0.08

[1]Control ewes (n = 10) were administered 0 mg/kg BW KNO_3
[2]NO_3^- treated ewes (n = 15) were administered 175 mg/kg BW KNO_3
[ab]Within a row, means with different superscripts differ ($P < 0.05$)

Discussion

Though weekly BW change did not differ (P = 0.56; Figure 1), control ewes were consistently numerically heavier than NO_3^- treated ewes. Previous studies in our laboratory (in review, 2008) showed that subacute dietary NO_3^- has little to no effect on weekly BW change during a short term treatment. Furthermore, gut fill can influence BW change according to the time of the animal's last meal in relation to when the BW was obtained (Rohr and Daenicke, 1984). Weekly BW was consistently recorded during the same time period in order to help eliminate weight variations from gut fill, though animal grazing time could not be controlled.

Plasma urea N is used as indicator of circulating N levels and glomerular filtration. Plasma urea N levels tended to be higher (P = 0.08; Figure 2) in NO_3^- treated ewes than controls. The increase in PUN levels in treated ewes was expected as the excess NO_3^- would increase circulating N levels; therefore, glomerular filtration rate would increase in an attempt to eliminate the toxic substance.

As expected, plasma NO_3^- levels were higher (P < 0.0001; Figure 3) in NO_3^- treated ewes. However, plasma NO_2^- was not different (P = 0.54; Figure 4) between the treatment groups. It is hypothesized this may be due to the NO_2^- binding to hemoglobin or being metabolized to other substrates. Another possible hypothesis was that the metabolism of NO_3^- to NO_2^- may have been slower in sheep than the three hour peak as seen in cattle, and therefore the time of blood collection was not conducive for detecting peak levels of NO_2^- production.

Decreased NH_4 production has been implicated in fertility problems in cattle and sheep. Plasma NH_4 levels did not differ (P = 0.64; Figure 5) among NO_3^- treated ewes compared to controls, indicating NH_4 levels may not be affected by subacute doses of dietary NO_3^-. An acute or chronic dose, however, may affect NH_4 levels. Laven et al. (2002) showed that quickly digestible N can increase plasma NH_4 and urea concentrations in ruminants.

It has been suggested that serum P_4 concentrations during the luteal phase are decreased when animals are administered high levels of NO_3^- over an 8 wk period due to luteal P_4 suppression (Page et al., 1990). Luteal P_4 synthesis may be decreased as a portion of the P-450 dependent hydroxylating enzymes have been inactivated by NO_3^- (Page et al., 1990). Progesterone levels were not affected (P = 0.57; Figure 6) by subacute dietary NO_3^- when administered prior to breeding. Therefore, a chronic dose of dietary NO_3^- may more severely impact circulating P_4 levels, inhibiting a ewe's ability to become pregnant or maintain a pregnancy.

Corpora lutea numbers were different (P = 0.001) in NO_3^- treated ewes as compared to controls (Table 1); however, fetuses counted using ultrasound did not differ (P = 0.14). A possible explanation for the difference noted in CL numbers but no difference in fetal counts by ultrasound is that counted CLs could be a result of pregnancy or ovulation. Furthermore, it is difficult to obtain precise fetal numbers by ultrasound at d 70 gestation due to fetus size. Stillborn lambs were defined as those that lived < 1 hr; a live birth was any lamb that lived > 1 hr. There were no differences (P > 0.33) in the number of stillborn lambs and live births between control and NO_3^- treated ewes. Interestingly, NO_3^- treated ewes gestation period tended (P = 0.08) to be shorter than control ewes, and NO_3^- treated ewes consistently had more difficulty during parturition.

Conclusion

In conclusion, fertility rates are not affected in ewes administered subacute dietary NO_3^-, two estrus cycles prior to breeding. Previous and current research have been unable to reproduce the reproductive complications associated with high levels of dietary NO_3^-, which implies that NO_3^- toxicity may not directly affect fertility Symptoms associated with subacute NO_3^- toxicity include lack of appetite, feed inefficiency, and immune suppression, which could be a greater problem to a developing embryo than the NO_3^- itself. Further research into suppression of the immune response and malnutrition and their effects on embryonic development may provide needed information as to how NO_3^- indirectly affects fertility.

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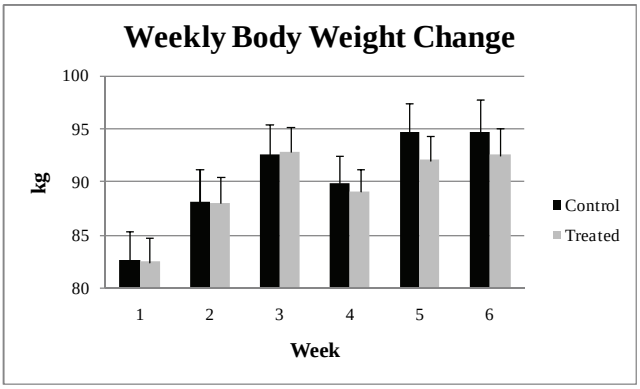

Figure 1. Weekly BW change did not differ ($P = 0.56$) between control and NO_3^- treated ewes.

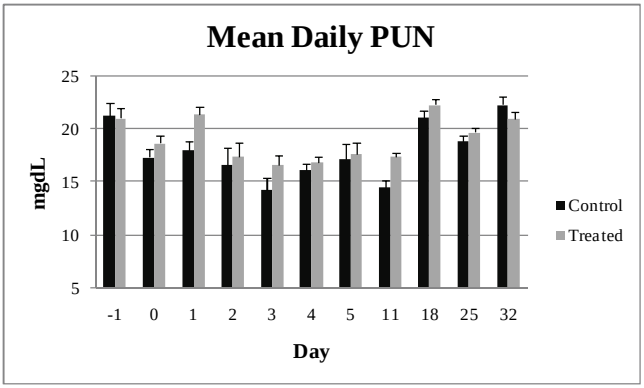

Figure 2. Plasma urea N tended to differ ($P = 0.08$) between control and NO_3^- treated ewes.

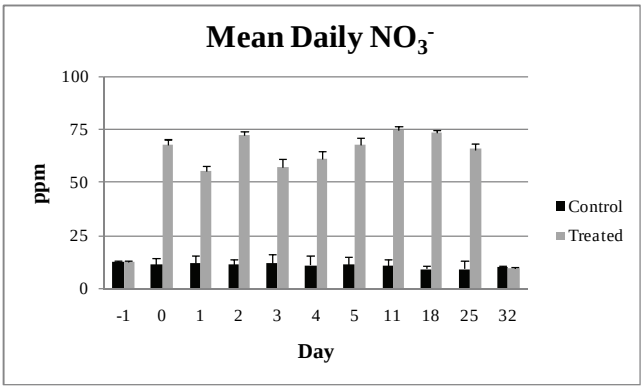

Figure 3. Circulating plasma NO_3^- was higher ($P < 0.0001$) in NO_3^- treated ewes than controls during the treatment period.

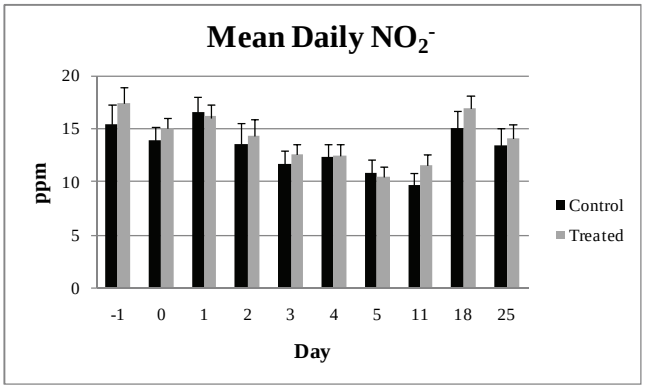

Figure 4. Circulating plasma NO_2^- did not differ ($P = 0.54$) between NO_3^- treated ewes and controls.

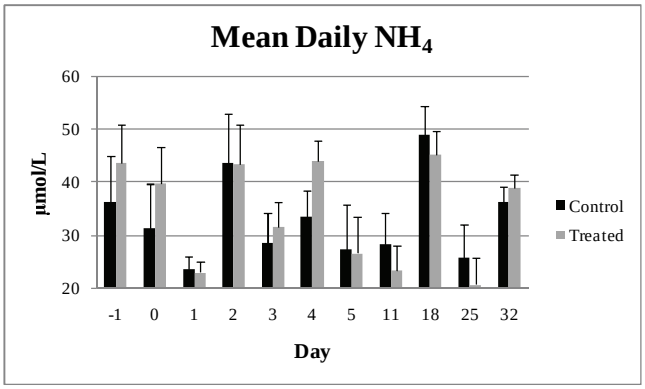

Figure 5. Circulating plasma NH_4 did not differ ($P = 0.64$) between NO_3^- treated ewes and controls.

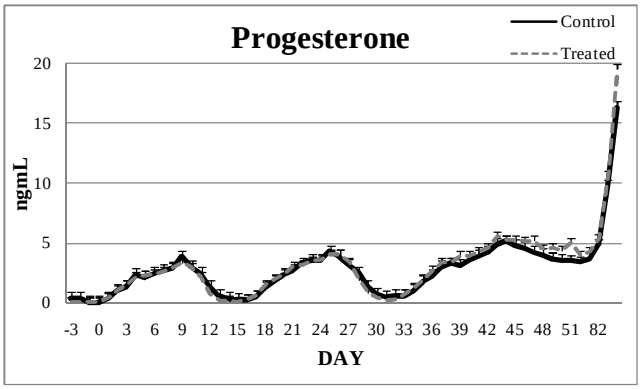

Figure 6. Daily circulating plasma P_4 levels did not differ ($P = 0.57$) between NO_3^- treated ewes and controls.

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INFLUENCE OF THIAMIN SUPPLEMENTATION ON FEEDLOT PERFORMANCE, CARCASS QUALITY, AND INCIDENCE OF POLIOENCEPHALOMALACIA IN LAMBS FED A 60% DISTILLERS DRIED GRAINS WITH SOLUBLES FINISHING RATION[1]

B. W. Neville[*], C. S. Schauer[†], K. Karges[‡], M. L. Gibson[‡], M. M. Thompson[†], P. T. Berg[*], and G. P. Lardy[*]

[*]North Dakota State University, Department of Animal Sciences, Fargo, ND 58105

[†]North Dakota State University, Hettinger Research Extension Center, Hettinger, ND 58639

[‡]Dakota Gold Research Association, Sioux Falls, SD 57104

ABSTRACT: Limited data are available regarding the influence of thiamin supplementation on incidence of polioencephalomalacia (**PEM**) in lambs fed diets containing high S levels (> 0.7%). Therefore, our objective was to evaluate the influence of thiamin supplementation on feedlot performance, carcass quality, and incidence of PEM in lambs fed a finishing diet containing 60% distillers dried grains with solubles (**DDGS**). Two studies (Study 1, 16 pens, 240 lambs; Study 2, 55 individually fed lambs) were conducted using completely random designs to evaluate the influence of level of thiamin supplementation. Lamb finishing diets contained 60% DDGS which resulted in dietary S concentration of 0.7% (DM basis). Treatment diets were based on level of thiamin supplementation, 1) **CON** (no supplemental thiamin), 2) **LOW** (50 mg·hd^{-1}·d^{-1}), 3) **MED** (100 mg·hd^{-1}·d^{-1}), or 4) **HIGH** (150 mg·hd^{-1}·d^{-1}). Additionally in Study 2, a fifth treatment (**HIGH+S**) was added which contained 0.87% S (DM basis) and provided 150 mg·hd^{-1}·d^{-1} thiamin. This increase in S was achieved by addition of dilute sulfuric acid to DDGS. In study 1, ADG changed quadratically (P = 0.04) with lambs fed CON, LOW, and MED gaining faster than lambs fed HIGH. In Study 1, DMI and G:F responded cubically ($P \leq$ 0.03) to level of thiamin supplementation with MED lambs having greater DMI and decreased G:F. No differences in performance data were observed in Study 2. In both studies, most carcass characteristics were unaffected with the exception of carcass conformation (Study 1; P = 0.05) and flank streaking (Study 2; P = 0.03). No clinical cases of PEM were observed during the course of either study. These data indicate limited benefits for the use of thiamin to aide in the prevention of PEM in lambs fed diets containing 60% DDGS and greater than 0.7% S.

Keywords: Lambs, Sulfur, Thiamin

Introduction

One of the challenges with use of ethanol co-products is the potential for high dietary S levels. High S diets can cause polioencephalomalacia (**PEM**) in ruminants. Inclusion of large percentages of co-product feeds, like distillers dried grains with solubles (**DDGS**), in finishing rations has been avoided, in part, due to problems with PEM as well as concerns about optimal animal performance and carcass characteristics. While the common dogma is that including DDGS at over 40% of dietary DM in beef cattle finishing diets will decrease performance, research indicates sheep can be fed higher levels of DDGS without affecting animal performance (Schauer et al., 2008). This provides an opportunity for increased utilization of DDGS in lamb finishing rations. Concerns remain if increased S levels in DDGS-based rations will result in PEM, and if a method of reducing or preventing PEM exists. Thiamin supplementation is one proposed method of reducing or preventing PEM in ruminant animals. The efficacy of thiamin supplementation in preventing PEM is likely impacted by the mechanisms by which PEM is caused (e.g. long-term thiamin deficiency or high hydrogen sulfide gas concentration). Further, the effect and dose of thiamin necessary to prevent such cases of PEM requires more investigation. For the purposes of this research, our hypothesis was that providing increased dietary thiamin would decrease the incidence of PEM in lambs fed high S diets without affecting animal performance. Therefore, our objectives were to determine the influence of thiamin level on feedlot performance, carcass characteristics, DMI, and incidence of PEM in lambs fed a 60% DDGS finishing ration.

Materials and Methods

Study 1. Prior to initiation of the research, all procedures were approved by the NDSU Animal Care and Use Committee. Two-hundred forty western white-face lambs (32.5 ± 4.8 kg; wethers and ewes) were utilized in a completely random design to evaluate the influence of level of thiamin supplementation in lamb finishing diets. The final finishing diet was balanced to contain 60% DDGS (DM basis), which resulted in a dietary S concentration averaging 0.72% (Table 1). Treatments diets differed in the amount of supplemental thiamin supplied; diets were formulated to provide: 1) **CON** (no supplemental thiamin), 2) **LOW** (50 mg·hd^{-1}·d^{-1} thiamin), 3) **MED** (100 mg·hd^{-1}·d^{-1}

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thiamin), or 4) **HIGH** (150 mg·hd^{-1}·d^{-1} thiamin) based on an estimated daily DMI of 1.36 kg·hd^{-1}·d^{-1}. Rations were mixed in a grinder-mixer and provided ad-libitum via bulk feeders. Contents of feeders (feed refusals) were collected and weighed at the end of the study. Initial and final weights were the average of 2-d weights. Following the 110-d finishing period, lambs were transported for harvest and subsequent carcass data collection at Iowa Lamb Corporation Hawarden, IA by trained personnel. One-hundred eighty-five lambs of the original 240 (77.08%) were shipped. Lambs with a live weight less than 50 kg 28 d prior to slaughter were not shipped to this location and as a result carcass data were not collected on these lambs. Treatment distribution of the lambs shipped to Iowa Lamb Corporation was 49, 48, 44, and 44 head for CON, LOW, MED, and HIGH, respectively.

Table 1. Ingredient and nutritional composition (DM basis) of final finishing ration fed to lambs in Study 1

	Treatments[1]			
Item	CON	LOW	MED	HIGH
Ingredient, %				
Alfalfa Hay	15.00	15.00	15.00	15.00
Corn	21.38	21.38	21.38	21.38
DDGS	60.00	60.00	60.00	60.00
Supplement[2]	3.62	3.62	3.62	3.62
Nutrient [3]				
CP, %	23.7	23.3	23.4	23.6
ADF, %	10.5	10.5	10.9	11.1
S, %	0.74	0.69	0.71	0.72
Ca, %	1.33	1.59	1.17	1.08
P, %	0.68	0.69	0.70	0.72
Thiamin[4]	0	50	100	150

[1] Treatments: CON (no supplemental thiamin), LOW (50 mg·hd^{-1}·d^{-1} thiamin), MED (100 mg·hd^{-1}·d^{-1} thiamin), and HIGH (150 mg·hd^{-1}·d^{-1} thiamin).
[2] Supplement (% total diet): 0.5% Ammonium chloride, 2.25% limestone, 0.085% Lasalocid, 0.78% Sheep Mineral 12 (Hubbard Feeds, Mankato MN), 0.002% Copper sulfate, and either 0, 0.004, 0.007, or 0.11% thiamin mononitrate.
[3] Laboratory analysis of nutrient concentration.
[4] Formulated level (ppm), thiamin inclusion in diet calculated based on an estimated DMI of 1.36 kg·hd^{-1}·d^{-1}.

Study 2. Fifty-five western white-face wether lambs (38.4 ± 3.2 kg) were utilized in a completely random design to evaluate the influence of level of thiamin supplementation and increased S level in lamb finishing diets containing 60% DDGS (treatment diets for CON, LOW, MED, and HIGH previously described, study 1). Additionally, a fifth treatment was added in which dietary thiamin was supplemented at the HIGH level while dietary S was increased from 0.71% to 0.87% (DM basis) with the addition of dilute sulfuric acid to DDGS (**HIGH+S;** Table 2). The number of lambs on each treatment was: 12, 10, 10, 12, and 11 head for CON, LOW, MED, HIGH, and HIGH+S, respectively. Lambs were assigned to one of five treatment diets and fed in individual pens for 112 d. Feed was offered daily and refusals were collected and weighed weekly. Initial and final weights were the average of 2-d weights. Following the 112-d finishing period, lambs were harvested and carcass data collected at the NDSU Meats Laboratory by trained personnel.

Table 2. Ingredient and nutritional composition (DM basis) of final finishing rations fed to lambs in Study 2

	Treatments[1]				
Item	CON	LOW	MED	HIGH	HIGH+S
Ingredient,%					
Alfalfa Hay	15.00	15.00	15.00	15.00	15.00
Corn	21.38	21.38	21.38	21.38	21.38
DDGS	60.00	60.00	60.00	60.00	60.00
Supplement[2]	3.62	3.62	3.62	3.62	3.62
Nutrient[3]					
CP, %	23.3	23.6	23.4	22.7	23.5
ADF, %	10.8	11.0	11.6	11.6	11.3
S, %	0.76	0.69	0.75	0.71	0.87
Ca, %	1.55	1.42	1.65	1.66	1.77
P, %	0.79	0.81	0.92	0.91	0.87
Thiamin[4]	0	50	100	150	150

[1] Treatments: CON (no supplemental thiamin), LOW (50 mg·hd^{-1}·d^{-1} thiamin), MED (100 mg·hd^{-1}·d^{-1} thiamin), HIGH (150 mg·hd^{-1}·d^{-1} thiamin), and HIGH+S (150 mg·hd^{-1}·d^{-1} thiamin with 0.87% S).
[2] Supplement (% total diet): 0.5% Ammonium chloride, 2.25% limestone, 0.085% Lasalocid, 0.78% Sheep Mineral 12 (Hubbard Feeds, Mankato MN), 0.002% Copper sulfate, and either 0, 0.004, 0.007, or 0.11% thiamin mononitrate.
[3] Laboratory analysis of nutrient concentration.
[4] Formulated level (ppm), thiamin inclusion in diet calculated based on an estimated DMI of 1.36 kg·hd^{-1}·d^{-1}.

Statistical Analysis. Lamb performance and carcass data were analyzed as a completely random design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NY) with pen (Study 1) and lamb (Study 2) serving as the experimental unit. Carcass data for Study 1 was analyzed with missing data points from underweight lambs not included in the data set, but with pen still serving as experimental unit. For both studies, the model included treatment while linear, quadratic, and cubic contrasts for increasing level of thiamin supplementation as well as a direct comparison of the HIGH vs. HIGH+S treatments (Study 2) were evaluated. *P*-values < 0.05 were considered significant and values less than 0.10 and greater than 0.05 were considered tendencies. When an overall F-test was not significant, but a contrast *P*-value was significant the results will be discussed as a tendency.

Results

Study 1. There was a tendency for quadratic ($P = 0.08$; Table 3) decrease in final BW; specifically the CON, LOW, and MED treatment lambs finished at heavier weights than the group fed the HIGH level of thiamin. This coincides with ADG which also exhibited a quadratic decrease ($P = 0.04$) with the CON, LOW, and MED treatment groups gaining weight at a faster rate than the HIGH treatment

group. Dry matter intake as well as G:F responded cubically ($P \leq 0.03$) to level of thiamin supplementation with the MED fed lambs consuming more feed resulting in decreased G:F.

Mortality was not affected ($P = 0.43$) by level of supplemental thiamin and averaged 0.42% across all treatments. Hot carcass weight tended to decrease quadratically ($P = 0.05$), while leg score had a quadratic tendency ($P = 0.06$) for a lower score with increased thiamin supplementation. Fat depth, body wall thickness, ribeye area, flank streaking, quality grade, and yield grade were all unaffected ($P \geq 0.17$) by level of supplemental thiamin. However, there was a cubic tendency ($P = 0.07$) for differences in conformation score with CON and MED having greater scores than LOW or HIGH. Additionally, lambs in the HIGH group tended to have a greater percentage of boneless closely trimmed retail cuts ($P = 0.06$; cubic response).

Study 2. There were no differences ($P \geq 0.46$; Table 4) in initial BW, final BW, ADG, DMI, leg score, carcass conformation, fat depth, body wall thickness, ribeye area, yield grade, or percentage boneless closely trimmed retail cuts of the lambs in Study 2. Gain efficiency and HCW tended differ in a cubic fashion ($P \leq 0.08$). Specifically, the lambs in the MED group had poorer G:F and lower HCW than all other treatment groups. There was a difference in flank streaking between the HIGH and the HIGH+S groups (325 vs. 482, respectively for HIGH and HIGH+S; $P = 0.002$). The increase in flank streaking further resulted in a tendency ($P = 0.02$) for differences in quality grade between HIGH and HIGH+S treatment groups. No differences in the incidence of morbidity or mortality were noted in Study 2 as no lambs died or were treated for illness during the study.

Discussion

The decrease in final weight with increasing level of thiamin in Study 1 was an unexpected result. Given that excess thiamin is cleared by the kidneys (McDowell, 2000) and that intake of upwards of 1000 times requirement are thought to be safe (NRC, 1987), it is difficult to attribute the decreased performance to thiamin toxicity at the levels fed in the present study. Palatability differences due to the sulfurous odor and bitter taste (McDowell, 2000) associated with thiamin could be another possible explanation for the differences in intake. Results from Study 2 contradict Study 1, as there were no differences in final BW among the treatments. Differences in HCW and leg score are more than likely driven by the similar differences observed in final BW in Study 1.

There were no occurrences of PEM observed during either of these studies; even though dietary S levels (0.69 to 0.87% S DM basis) were nearly twice the recommended maximum tolerable level of S (0.4% for high concentrate diets; NRC, 2005). Contrary to the present studies, Krasicka et al. (1999) reported that all lambs fed a low fiber-high starch diet containing 0.72% S died from PEM after 12 weeks. Loneragan et al. (2005) hypothesized that the therapeutic effects of thiamin in PEM-affected animals are either due to an increased requirement for thiamin or a beneficial effect of thiamin on impaired brains. The present research discounts the proposed increased requirement at least in feedlot lambs fed high levels of DDGS as the S source. However, we cannot support or dismiss the second theory relating to the beneficial effect of thiamin on impaired brains as no clinical cases of PEM occurred in our studies. Our data suggests that PEM cannot be induced in lambs fed 0.69 to 0.87% S when the primary dietary S source is DDGS. This indicates the threshold level may be greater in lambs than previously thought, or that disposition of S (what form it is in the feed, its fate during ruminal fermentation, and its route of excretion) should be investigated more fully.

A review of literature reporting the amount of S fed to ruminants in corn co-product-based rations further demonstrates the inconsistencies in the amount of S required to cause neurological problems, such as PEM. The present study demonstrates that feeding DDGS at 60% of dietary DM does not appear to increase the incidence of PEM in lambs when water with low sulfate content (< 141 ppm) is available. Similar to the present studies the 60% DDGS diet fed by Schauer et al. (2008) contained 0.55% S (DM basis) did not result in any cases of PEM. Contrary to these studies Niles et al. (2002) reported that 10 of 14 calves fed corn gluten feed-based diets exhibited PEM; those calves affected were fed diets that contained either 0.55 or 0.70% S (DM basis). Huls et al. (2008) fed 50% modified wet distillers grains plus solubles while supplementing 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin without inducing PEM; while Buckner et al. (2007) discontinued feeding a treatment diet which contained 50% DDGS when multiple steers exhibited PEM while receiving 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin.

Further, our data indicates the NRC (2005) maximum tolerable level of S should be re-evaluated. At a minimum, Schauer et al. (2008) and the present studies illustrate the need for additional research to further determine the interactive affects of S, thiamin supplementation, and dietary grain concentration in finishing rations; and the effect they collectively have on the incidence of PEM. Further investigation into S metabolism in lambs as well as determining if beef cattle can be fed similar levels of S with out negative impacts on performance and health will be beneficial for not only livestock producers but also the ethanol industry.

Implications

The fact that lambs fed diets averaging 0.72% sulfur did not develop polioencephalomalacia while still maintaining adequate performance, even when given no supplemental thiamin demonstrates that feeding elevated levels of distillers dried grains with solubles is possible in lamb finishing diets. The use of thiamin as a dietary additive to aide in the prevention of polioencephalomalacia in finishing lambs does not appear to be necessary in feeding environments with similar feed and water sulfur levels as the present studies. Additionally, feeding 150 $mg \cdot hd^{-1} \cdot d^{-1}$ thiamin appears to prevent polioencephalomalacia in lambs fed diets containing 0.87% sulfur.

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Table 3. Influence of thiamin supplementation on performance and carcass characteristics of lambs in Study 1

	Treatment[1]						*P*-value[3]		
Item	CON	LOW	MED	HIGH	SEM[2]	*P*-value	Linear	Quad	Cubic
Initial wt, kg	32.6	32.6	32.5	32.6	0.15	0.94	0.73	0.63	0.83
Final wt, kg	62.3	62.8	62.5	60.5	0.65	0.10	0.07	0.08	0.79
ADG, kg/d	0.27	0.28	0.27	0.25	0.005	0.08	0.09	0.04	0.76
Intake, kg·hd^{-1}·d^{-1}	1.77	1.78	1.98	1.74	0.04	0.001	0.49	0.004	0.002
G:F	0.15	0.15	0.14	0.15	0.004	0.05	0.08	0.57	0.03
Mortality, %	1.67	0	0	0	0.83	0.43	0.20	0.34	0.66
HCW, kg	31.4	32.1	31.7	30.9	0.37	0.18	0.35	0.05	0.68
Leg score[4]	11.3	11.5	11.6	11.0	0.17	0.16	0.36	0.06	0.41
Conformation score[4]	11.5	11.4	11.6	11.2	0.08	0.05	0.07	0.12	0.07
Fat depth, cm[5]	0.8	0.9	0.8	0.8	0.05	0.59	0.96	0.96	0.18
Body wall thick, cm	2.7	3.0	2.5	2.7	0.10	0.32	0.39	0.83	0.11
Ribeye area, cm^2	15.6	15.5	15.7	15.7	0.39	0.98	0.77	0.92	0.81
Flank streaking[6]	337	340	353	336	6.74	0.29	0.71	0.16	0.21
Quality grade[4]	11.3	11.3	11.5	11.2	0.08	0.17	0.36	0.13	0.15
Yield grade[7,5]	3.5	3.8	3.4	3.7	0.18	0.55	0.82	0.94	0.17
%BCTRC[8]	44.7	44.3	45.0	46.8	0.21	0.18	0.24	0.75	0.06

[1] Treatments: CON (no supplemental thiamin), LOW (50 mg·hd^{-1}·d^{-1} thiamin), MED (100 mg·hd^{-1}·d^{-1} thiamin), and HIGH (150 mg·hd^{-1}·d^{-1} thiamin).

[2] Standard Error of Mean; n = 4.

[3] *P*-value for linear, quadratic, and cubic effects of increasing level of thiamin supplementation.

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

[5] Adjusted fat depth and yield grades.

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

[7] Yield Grade = 0.4 + (10 x adjusted fat depth).

[8] % Boneless closely trimmed retail cuts = (49.936 - (0.0848 x HCW, lbs) - (4.376 x fat depth, in) - (3.53 x body wall thickness, in) + (2.456 x ribeye area, in^2)).

Table 4. Influence of thiamin supplementation and added sulfur on performance and carcass characteristics of lambs in Study 2

	Treatment[1]						*P*-value[3]			
Item	CON	LOW	MED	HIGH	HIGH+S	SEM[2]	*P*-value	Linear	Quad	Cubic	HIGH vs. HIGH+S
Initial wt, kg	38.5	38.7	38.4	38.1	38.8	1.30	0.99	0.79	0.85	0.96	0.70
Final wt, kg	59.7	61.0	58.1	60.7	60.9	1.72	0.74	0.99	0.69	0.22	0.94
ADG, kg/d	0.19	0.20	0.18	0.20	0.20	0.01	0.48	0.76	0.46	0.11	0.77
Intake, kg·hd^{-1}·d^{-1}	1.30	1.30	1.24	1.27	1.30	0.05	0.86	0.44	0.70	0.49	0.68
G:F	0.15	0.15	0.14	0.16	0.15	0.005	0.17	0.15	0.32	0.07	0.20
HCW, kg	30.6	31.8	29.3	30.7	31.5	0.97	0.40	0.60	0.90	0.08	0.56
Leg score[4]	11.1	11.1	10.8	11.0	11.2	0.22	0.78	0.55	0.67	0.41	0.54
Conformation score[4]	10.8	10.9	10.7	10.8	11.1	0.19	0.56	0.80	0.79	0.48	0.18
Fat depth, cm[5]	0.8	0.7	0.7	0.7	0.9	0.10	0.60	0.71	0.35	0.99	0.35
Body wall thick, cm	2.6	2.4	2.4	2.5	2.7	0.13	0.46	0.37	0.36	0.99	0.21
Ribeye area, cm^2	14.4	14.1	13.7	14.9	14.3	0.58	0.70	0.62	0.21	0.56	0.45
Flank streaking[6]	392	370	350	325	482	37.25	0.03	0.16	0.96	0.97	0.002
Quality grade[4]	11.3	11.1	11.1	11.0	11.7	0.22	0.12	0.41	0.91	0.80	0.02
Yield grade[7,5]	3.5	3.1	3.1	3.3	3.8	0.36	0.60	0.71	0.35	0.99	0.35
%BCTRC[8]	44.7	44.7	45.2	45.1	44.3	0.47	0.59	0.35	0.93	0.69	0.16

[1] Treatments: CON (no supplemental thiamin), LOW (50 mg·hd^{-1}·d^{-1} thiamin), MED (100 mg·hd^{-1}·d^{-1} thiamin), HIGH (150 mg·hd^{-1}·d^{-1} thiamin), and HIGH+S (150 mg·hd^{-1}·d^{-1} thiamin with 0.87% S).

[2] Standard Error of Mean n = 12, 10, 10, 12, and 11 head for CON, LOW, MED, HIGH, and HIGH+S respectfully.

[3] *P*-value for linear, quadratic, and cubic effects of increasing level of thiamin supplementation; as well as direct comparison of HIGH and HIGH+S treatments.

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

[5] Adjusted fat depth and yield grades.

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

[7] Yield Grade = 0.4 + (10 x adjusted fat depth).

[8] % Boneless closely trimmed retail cuts = (49.936 - (0.0848 x HCW, lbs) - (4.376 x fat depth, in) - (3.53 x body wall thickness, in) + (2.456 x ribeye area, in^2)).

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PREGNANCY RATE, OFFSPRING PERFORMANCE AND SERUM PROLACTIN, ESTRADIOL, INSULIN, AND GLUCOSE CONCENTRATIONS IN EWES TREATED WITH RECOMBINANT BOVINE SOMATOTROPIN BEFORE BREEDING

L. E. Camacho, J. M. Benavidez, and D. M. Hallford
New Mexico State University, Las Cruces, NM 88003

ABSTRACT: Rambouillet ewes were used to examine effects of bovine somatotropin (bST) near the time of breeding on serum hormone profiles and pregnancy rates. Before initiation of a fall breeding period, 75 ewes (68.8 ± 1.5 kg) received an intravaginal insert containing 0.3 g of progesterone (P4) to synchronize onset of estrus. After 12 d, inserts were removed (d 0) and ewes received either 0 (control, n = 37) or 250 (n = 38) mg of recombinant bST (Posilac, Monsanto, s.c.). Ewes were joined with fertile rams 24 h after insert removal. Blood samples were collected from 12 ewes in each treatment daily from d 0 to 20 after insert removal. As expected, serum IGF-I concentrations were elevated (P = 0.05) from d 2 through 13 in bST-treated compared with control ewes. Serum PRL and estradiol were similar (P > 0.97) between treatments. Serum insulin concentrations were 0.44 and 1.74 (± 0.19) ng/mL in control and bST-treated ewes 1 d after receiving bST (P = 0.001) and remained elevated (P < 0.03) in bST-treated ewes through d 9. Peak insulin values in bST-treated ewes (6.94 vs 0.39 ± 1.41 ng/mL for controls) occurred on d 7. Serum glucose values were 98 and 69 (± 5) mg/dL in bST-treated and control ewes 2 d after bST injection (P = 0.001). Treated ewes continued to have greater (P < 0.003) serum glucose concentrations than did control ewes until d 8 at which time values were 108 and 55 (± 12) mg/dL (P = 0.004), respectively. Pregnancy rates were determined at lambing. Thirty-three of 37 (89%) control ewes were pregnant whereas 27 of 38 (71%) bST-treated ewes were pregnant (P = 0.05). As a percentage of ewes lambing, 61% and 39% of control ewes produced single and twin lambs, respectively, compared with 41 and 59% of bST-treated ewes (P = 0.12). Lamb 60-d adjusted weaning weights were 23.0 and 21.2 (± 0.65) kg for offspring produced by control and bST-treated dams, respectively (P = 0.04). Pregnancy rates and offspring weights were decreased by bST treatment immediately before breeding. However, if bST-treated ewes conceived, they tended to produce more twins.
Keywords: growth hormone, reproduction, sheep

Introduction

Establishment of pregnancy depends on an appropriate relationship between dam and embryo. Maternal environment may not always be appropriate (Wilmut et al., 1985). Thatcher et al. (2001) showed that bovine somatotropin (**bST**) affects the maturing oocyte, reproductive tract, or developing embryo to enhance embryonic survival. Bovine somatotropin improves fertilization rate, accelerates embryo development, and improves embryo quality (Santos et al., 2004). Early embryonic loss is a main cause of reproductive failure, particularly in repeat-breeding cows, where close to 50% of embryos die during the first 16 d after fertilization (Morales-Roura et al., 2001). In sheep, 20 to 30% of embryos are lost during the first 13 d after fertilization (Carrillo et al., 2006). Bilby et al. (2004) suggested that bST may enhance conceptus development, allowing greater secretion of interferon tau at d 17 of pregnancy, and also, may increase number of pregnant animals and decrease early embryonic loss. Morales-Roura et al. (2001) suggested that bST could reduce delayed embryo development as it increases serum progesterone (**P4**) and delays onset of luteolysis in heifers. Growth hormone may improve embryo development in cattle; its addition to culture media stimulates *in vitro* oocyte maturation and subsequent embryonic development (Morales-Roura et al., 2001). Carrillo et al. (2006) found that a single administration of bST 5 d before exogenous progestin withdrawal increased the proportion of ewes with multiple pregnancies, lambing rate of mature ewes, and prolificacy. Effects were associated with an increase in serum IGF-I. The objective of this experiment was to determine the influence of recombinant bST administered to ewes immediately before estrus on pregnancy rates, offspring performance, and serum concentrations of prolactin (**PRL**), estradiol, insulin, and glucose.

Materials and Methods

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

Animals and Treatments. Seventy five mature Rambouillet ewes (68.8 ± 1.5 kg) were maintained under ambient conditions during a fall breeding season at the West Sheep Unit on the main campus at New Mexico State University. Ewes were fed alfalfa hay at 1.6 kg daily and had free access to water, salt, and shade. Before initiating the fall breeding period, ewes received a P4-impregnated intravaginal insert (**CIDR**, 0.3 g P4; Pharmacia and Upjohn Pty Limited, Rydalmere NSW) to synchronize onset of estrus. The CIDR was removed after 12 d and ewes were joined with fertile Rambouillet rams. Ewes were stratified by BW and age and randomly assigned to 1 of 2 treatments on the day of CIDR removal (d 0). Thirty seven control ewes received a s.c. injection containing 0.5 mL of saline immediately after CIDR removal. The second group of 38 ewes received 250 mg of prolonged release bST (Posilac, Monsanto Co.).

Blood Collection and Analysis. Beginning on d 0 and continuing through d 20, blood was collected daily from 12 ewes in each treatment by jugular venipuncture into serum separator tubes (Corvac, Kendall Health Care, Sr. Louis, MO) and allowed to clot at room temperature for 30 min. Samples were centrifuged at 4°C for 15 min at 1,500 x g and serum was stored frozen in plastic vials until assayed. Serum IGF-I (Berrie et al., 1995), PRL (Spoon and Hallford, 1989), and estradiol (Kane et al., 2004) were quantified by double antibody RIA. Insulin (Riemers et al., 1982) was quantified by solid phase RIA using components of commercial kits (Coat-A-Count Siemens Medical Solutions Diagnostics; Los Angeles, CA). Within and between assay CV were less than 15% for all hormone determinations.

Serum glucose was determined using a glucometer (One Touch UltraMini, Life Scan, Johnson and Johnson, Milpitas, CA). Briefly, the glucometer method employs test strips (One Touch Ultra Test Strip) which contain glucose oxidase (*Aspergillus niger*) and ferricyanide. Approximately 10 µL of serum was placed on the test strip and the strip was placed in the glucometer which yielded a glucose reading in about 5 s. The performance characteristics provided with the glucometer and test strips stated the reference range was 20 to 600 mg/dL. To validate the test in ruminant serum, 59 samples were tested by both colorimetric (enzymatic endpoint method, # TR 12421, Thermo Scientific Waltham, MA) and glucometer methods. The mean glucose concentration determined by the 2 methods did not differ (70 and 76 ± 3 mg/dL for colorimetric and glucometer, respectively, $P = 0.16$) and the correlation coefficient between methods was 0.70 ($P < 0.001$). All samples were assayed on 1 d and the CV determined by testing 1 sample 8 times was 3.7%.

Statistical Analysis. Effects of bST on IGF-I, PRL, estradiol, insulin, and glucose during the treatment period were examined by split-plot analysis of variance using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The main plot factor was bST treatment and individual ewe was the experimental unit. Sampling day and the day by bST treatment interaction were included in the subplot. Compound symmetry was the appropriate covariance structure. When significant treatment by day interactions were detected, treatment effects were examined within day of sampling. Pregnancy rate was examined using the frequency procedure of SAS. Weight responses were subjected to ANOVA for a completely random design and analysis was computed using GLM of SAS.

Results and Discussion

Pregnancy Rates and Offspring Performance

As stated previously, one of the objectives of this experiment was to determine the influence of bST on pregnancy rate of ewes. Our interest was to elevate the serum concentration of IGF-I during the time of ovulation, breeding, and early embryonic development. In a preliminary report from our laboratory, Camacho et al. (2008) stated that serum IGF-I concentrations were elevated in bST-treated ewes compared with controls from 2 to 13 d after bST administration. Similar increases in IGF-I in response to bST treatment were reported by Carrillo et al. (2006) in Pelibuey ewes. Pregnancy rates in the current study were determined at lambing. Thirty-three of 37 (89%) control ewes were pregnant compared with 27 of 38 (71%) bST-treated ewes ($P = 0.05$). These data suggest a decrease in conception rate in Rambouillet ewes treated with 250 mg of bST at the time of CIDR removal. In contrast, pregnancy rates were increased in cyclic, lactating dairy cows when bST was injected at initiation of the Ovsynch protocol or near the time of AI (Moreira et al., 2000; Morales-Roura et al., 2001; Santos et al., 2004).

The distribution of control ewes producing 0, 1, or 2 lambs was 11, 54, and 35%, respectively, compared to 29, 29, and 42%, respectively, for bST-treated ewes ($P = 0.05$). However, when only ewes that became pregnant were examined, 61 and 39% of control ewes produced single and twin lambs, respectively, compared with 41 and 59% of bST-treated females, respectively ($P = 0.12$). These results suggest a tendency for bST-treated ewes to produce more twins if the ewe becomes pregnant. Likewise, Carrillo et al. (2006) reported that bST administration to ewes 5 d before CIDR removal increased number of lambs born compared with controls.

Offspring produced from these matings were weighted at birth and again when weaned at approximately 60 d of age. Lambs from control and bST-treated dams weighted 5.4 and 5.0 (± 0.12) kg, respectively, at birth ($P =0.01$). At weaning, offspring from control ewes weighted 20.9 kg compared with 18.9 (± 0.7) kg for those from bST-treated ewes ($P = 0.04$). As an additional variable, actual weaning weight was adjusted to a 60 d, single ewe lamb, mature ewe basis. Adjusted weaning weights were 23.0 and 21.2 (± 0.6) kg for offspring of control and bST-treated ewes, respectively, ($P = 0.04$). The decrease in birth weight of offspring from bST-treated ewes contrast data reported by Costine et al. (2005) who observed heavier birth weights of lambs from ewes treated with GH. The reason for decreased lamb growth response from bST-treated ewes is not readily apparent. It is doubtful that prebreeding administration of bST would adversely impact ewe milk production 5 or 6 mo later. In fact, Holcombe et al. (1988b) treated ewe lambs with oGH for 30 d before breeding and observed no differences among treatments in milk yield or composition. The possibility exists that bST administration before breeding could influence endocrine parameters that might alter postnatal lamb performance in addition to influencing conception rates of ewes.

Serum Hormone Profiles

Serum PRL was measured in samples collected daily from the time of CIDR and bST administration through the subsequent 20-d period. A treatment by day interaction was detected ($P = 0.032$) necessitating examination of treatment effects within sampling day. The PRL concentrations in control and bST-treated ewes did not differ ($P > 0.10$) on any day after treatment (data not shown). Flores et al. (2008) reported that serum PRL was positively correlated with diameter of the largest follicle 1 d after CIDR removal and $PGF_{2\alpha}$ administration in beef cows. However, these same workers stated that bST did not affect serum PRL concentrations.

Serum estradiol concentrations were also determined in the daily post-treatment samples (treatment by day, $P = 0.17$) and no differences were observed ($P > 0.12$) between treatments data not shown). This similarity in serum estradiol values in the 2 groups implies that this hormone was likely not a contributor to the decrease in conception rate observed in bST-treated ewes. Previous research by Bartholomeusz et al. (1999) in rats showed that small increases in maternal estradiol could be lethal to embryos and retard fetal and placental growth.

Serum insulin profiles in control and bST-treated ewes after CIDR removal and bST administration are shown in Figure 1 (treatment by day, $P < 0.001$). Before treatment on d 0, serum insulin values were less than 1.0 ng/mL in both groups. One day after treatments began, however, insulin in control ewes was 0.44 ng/mL compared with 1.74 (± 0.19) ng/mL in bST-treated females ($P < 0.001$). A single injection of bST resulted in very large increases in serum insulin such that on d 7 the value in treated ewes was 6.94 ± 1.41 ng/mL compared with 0.39 ng/mL in control ewes ($P = 0.003$). The significant increase in insulin for treated ewes continued to d 9 and remained numerically greater than controls through d 11. Holcombe et al. (1988ab) also observed increased serum insulin concentrations in mature ewes and ewe lambs treated daily with exogenous oGH. Schemm et al. (1990) suggested that exogenous GH decreased glucose clearance in response to insulin and that GH reduced the inhibition of gluconeogenesis by insulin. Growth hormone may be responsible for insulin resistance in maternal tissue but the mechanism is not known (Brockman and Laarveld, 1986). However, increasing concentrations of insulin in goats enhanced ovarian function and twinning percentage (Suguna et al., 2008).

After observing the large increase in serum insulin values in response to bST administration, serum glucose was determined in samples through d 12 and values are presented in Figure 2 (treatment by day, $P < 0.001$). Before treatment on d 0, glucose values were 43 ± 2.9 mg/dL in both groups. At 24 h after treatment, control ewes had a glucose concentration of 69 mg/dL compared with 98 (± 5.4) mg/dL for bST-treated ewes ($P < 0.001$). The peak glucose values in treated ewes (121 ± 12 mg/dL) occurred on d 6 at which time the value for control ewes was 53 ± 12 mg/dL ($P < 0.001$). Bell and Bauman (1997) suggested that exogenous bST can cause reduced glucose uptake in muscle and adipose tissue, reduction in insulin receptor protein abundance (inhibitory effect on GLUT4), and may increase mobilization of amino acids.

Implications

Pregnancy rates and offspring performance were decreased by bovine somatotropin treatment immediately before breeding. If somatotropin-treated ewes conceived, they tended to produce more twins. Treatment with somatotropin resulted in large increases in serum insulin and glucose concentrations which may have contributed to effects observed on reproduction and lamb weights.

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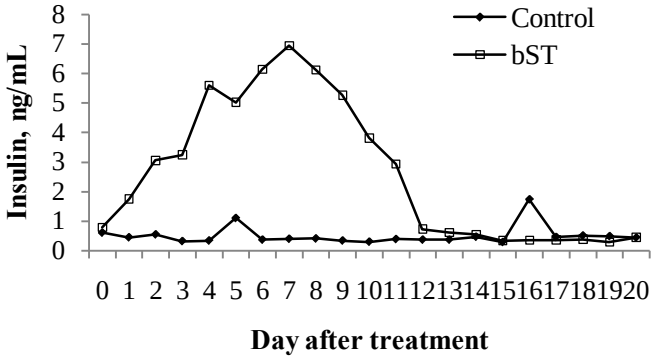

Figure 1. Serum insulin concentration in Rambouillet ewes treated with 0 (control, n = 11) or 250 mg (bST, n = 12) of bovine somatotropin (bST) beginning on the day of removal of a progesterone-containing intravaginal insert (d 0). Values differed between treatments on d 1 through 9 ($P < 0.03$). The SE ranged from 0.05 to 1.57 ng/mL.

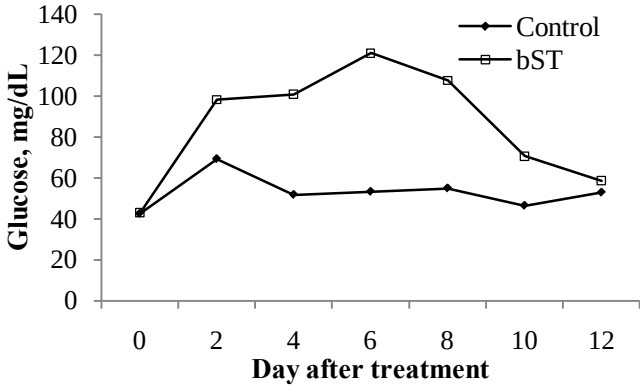

Figure 2. Serum glucose concentration in Rambouillet ewes treated with 0 (control, n = 11) or 250 mg (bST, n = 12) of bovine somatotropin (bST) beginning on the day of removal of a progesterone-containing intravaginal insert (d 0). Values differed between treatments on d 2 through 8 ($P < 0.008$). The SE ranged from 2 to 12 mg/dL.

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THE EFFECTS OF STARCH OR FIBER BASED SUPPLEMENTS ON DIGESTIBILITY AND FEEDING BEHAVIOR IN HEIFERS AND WETHERS

T. J. McDonald, J. A. Paterson, B. M. Nichols, and M. M. Harbac
Department of Animal & Range Sciences, Montana State University, Bozeman, MT 59717

ABSTRACT: The objectives of three experiments were to study the effects of starch (corn grain) or fiber (wheat middlings) based supplements on digestibility of DM, NDF and N by wethers and feeding behavior by heifers. In Exp. 1, 16 wethers were randomly assigned to chopped grass hay (8.9% CP) based diets containing 0, 5, 10, or 20% cracked corn (CORN) while in Exp. 2, the wethers were assigned to grass hay based diets containing 0, 5, 10, or 20% wheat middlings (MID). Each experiment consisted of 10 d diet adaptation followed by 5 d of total fecal collection.. Dry matter intakes were limit fed at 1.35 kg·wether^{-1}·d^{-1}. There were no ($P > 0.05$) changes measured for CP or DM digestibilities due to increasing CORN, but NDF digestibility decreased linearly ($P < 0.05$). In Exp. 2, digestibility of CP increased linearly ($P = 0.02$) with increasing MID, but NDF or DM digestibilities were not changed ($P > 0.05$). For Exp. 3, 24 Angus heifers (17-mo-old, BW = 366 kg, 2-mo-pregnant) were used in a completely randomized design to study the effects of CORN or MID supplements on feeding behavior. Heifers were assigned to 1 of 4 supplement groups; no supplement (HAY), 20% corn (CORN), 20% wheat middlings (MID), or a 50% corn and 50% wheat midds mix (20% of DMI; MIX). Heifers were assigned to one of eight pens with 3 heifers/pen and two pens/treatment for a 20 d diet adaptation followed by 10 d of data collection. Heifers were offered ad libitum access to diets (80% chopped grass hay and 20% supplement). Individual intakes and feeding behavior were measured using the GrowSafe® 4000 system. Numerically, total DM intake was greatest for MID and least for CORN ($P < 0.05$). However, intake during the first 6 h after feeding and the percentage of the total 24 h intake consumed during the first 6 h was greater for MIX ($P < 0.05$) than HAY, CORN, or MID. CORN depressed NDF digestion while MID did not, but heifers consumed 10% more DM when fed MIX during the first 6 h after feeding.

Key words: Corn supplementation, Feeding behavior, Nutrient digestion

Introduction

When dormant native range or hay does not provide enough protein or energy to meet the nutritional requirements of beef cattle, supplementation may be necessary to meet changing physiological status, environmental stress, or inadequate forage supply (Chase and Hibberd, 1987). Supplementing native grass hay diets with grain-based supplements continues to be a debatable topic among producers and researchers (Matejovsky and Sanson; 1995 and Sanson et al., 2004). Most literature suggests a decrease in neutral detergent fiber (NDF) digestibility with increasing levels of cereal grain supplementation (Chase and Hibberd, 1987, Sanson and Clanton, 1989). Martin and Hibberd (1990) found that energy supplementation with high fiber (soybean hulls) did not reduce hay NDF digestion. Work by Orr et al. (2008) recently showed that replacing a portion of the corn supplement with soybean hulls may actually improve fiber digestion. The effect of supplement source on feeding behavior has been compared with beef cattle. For example, Cooke et al. (2007) found greater variation in intake for a citrus pulp based supplement compared to a molasses based supplement.

The objectives of these three experiments were to measure the effects of increasing levels of fiber (wheat middlings) or starch (corn grain) based supplements on DM, NDF, and N digestibilies in wethers and secondly, how these supplements would influence feeding behavior in pregnant heifers.

Materials and Methods

Exp. 1 & 2

Sixteen crossbred wether lambs (Suffolk/Hampshire x Western white face; 6-mo-old; BW = 40 kg) were used in a completely randomized design to investigate the effects of increasing levels of starch (cracked corn grain; **CORN**) or fiber (wheat middlings; **MID**) based supplements on digestibility of DM, NDF, and N. Wethers were weighed, without overnight feed restriction, at the beginning and end of the experiment. They were housed in the Montana State University's Nutrition Center and in individual metabolism crates measuring 1.17 x 0.69 x 0.69 m. The facility was environmentally controlled with continuous lighting and temperature maintained above 20°C. Animal care and handling techniques were approved by the Montana State University Institutional Animal Care and Use Committee.

Four wethers were randomly assigned to each dietary treatment. Dry matter intakes were limit fed at 1.35 kg·wether^{-1}·d^{-1}. In Exp. 1, the diet consisted of chopped native grass hay (9.6% CP) supplemented with 0, 5, 10, or 20% CORN (8.4 % CP). In Exp. 2, chopped native grass hay (8.2% CP) was fed supplemented with 0, 5, 10, or 20% MID (17.7% CP). For both experiments, hay was chopped to a length of approximately 2.54 cm with an Art's Way Grinder/Mixer®. Wethers had continuous access to water and were fed daily at 1200.

Each experimental period consisted of 10 d diet adaptation period followed by five d of total fecal

collection. Canvas fecal collection bags were fitted to the animals starting on d 8 with fecal collection conducted from d 11 through d 15. Total fecal and feed samples were collected once daily and composited for later analysis. After the collection period, total fecal excretion was weighed and mixed for 3-min. A 200 g subsample was collected and dried at 60°C for 48 h to calculate partial DM. Diet and supplements were processed similarly. All samples were then ground to pass through a 1.0 mm screen and analyzed for neutral detergent fiber (NDF; Van Soest et al., 1991), and N (AOAC, 1999). All samples were also dried at 100°C for an additional 24 h to determine intakes and fecal excretion on a moisture free basis.

Statistical Analysis

Data was analyzed by nonlinear polynomial regression (Statistics 9, 2008) to determine if linear or quadratic responses existed when increasing amounts of CORN or MID supplements were added to the diet. Differences were considered significant at $P < 0.05$.

Exp. 3

Twenty-four Angus heifers (17-mo-old, BW= 366 kg, 2-mo-pregnant) were used in a completely randomized design to study the effects of CORN, MID or a 50% CORN and 50% MID supplement on DM intake and feeding behavior. Heifers were housed in feedlot pens at the Montana State University BART Farm. Heifers were randomly assigned to 1 of 8 pens (12.2 x 4.6 m) with 3 heifers per pen. Each pen contained a GrowSafe® 4000 feeder which allows for individual feed consumption using radio frequency ear tags (RFID) and feeding tubs placed on load cells and equipped with an antenna which allows for measurement of total feed intake and feeding behavior (intakes every minute, number of visits to the tub/day, etc).

The diet consisted of 80% chopped native grass hay (12% CP) and 20% supplement. Pens were randomly assigned to one of four supplement treatments (two pens per supplement group); no supplement (**HAY**), cracked corn (**CORN**), wheat middlings (**MID**), or a 50% corn and 50% wheat middlings mix (**MIX**). The grass hay was chopped to a length of 10 cm with a JAY•LOR Mixer/Feeder®. Heifers had continuous access to water and trace mineralized salt blocks. The diets were mixed daily in a Roto-Mix® TMR Mixer/Feeder and fed at 0800.

The experimental period consisted of a 20 d adaptation period followed by 10 d of measurement of total intake and feeding behavior (DM consumed during the first 6 h, time spent at the feed tub, and total time spent at the feed bunk during a 24 h period). Results were analyzed as a completely randomized design (Statistics 9, 2008) with animal as the experimental unit since individual feed intakes were measured. Means were separated by the LSD procedure and differences were considered significant at the $P < 0.05$ level.

Results and Discussion

In Exp. 1, digestibilities of DM or N were not different *($P > 0.05$)* among diets with increasing levels of CORN (Table 1). However, digestibility of NDF decreased linearly *($P < 0.05$)* with increasing CORN (Fig. 1). This result agrees with the linear decrease Chase, Jr. and Hibberd (1987) reported when beef cows were supplemented with 0, 1, 2, or 3 kg of cracked corn to low quality (4.2% CP) native grass hay. Feeding the higher corn levels decreased forage utilization to the extent that overall energy status of the cows was not improved. Sanson et al. (1990) also showed that hemicelluloses digestion decreased as the level of corn in the diet increased while Royes et al. (2001) found decreased NDF and ADF digestion as level of corn supplementation increased with ammoniated star grass.

In Exp. 2, digestibility of N increased linearly *($P = 0.02$)* with increasing MID (Table 2). However, there were no differences *($P > 0.05$)* due to increasing MID on NDF (Fig. 2) or DM digestibilities. Similarly, Martin and Hibberd (1990) found no differences when cows were supplemented with 0, 1, 2, or 3 kg/d of soybean hulls with a 4.1% CP native grass hay. Interestingly, Galloway et al., (1991) found that wheat middlings as a supplement to bermudagrass or ryegrass had a depressing effect on NDF digestion when measured with steers.

In Exp. 3, numerically *($P < 0.05$)* MID had the greatest intake (12.9 kg/d) and CORN had the lowest (11.5 kg/d; Table 2). Dry matter intake during the first 6 h after feeding was highest *($P < 0.05$)* for MIX (8.7 kg) but similar among the other treatments (avg. 5.8 kg). As a percentage of the total 24 h consumption, steers fed MIX consumed 73.3% of the total daily ration during the first six hours compared to the other treatments which averaged 44.8% of the total ration consumed during the first six hours. Orr et al. (2008) reported that replacing a portion of supplemental corn with soybean hulls may actually improve fiber digestion and N utilization. Grigsby et al.(1993) found similar results with steers fed 100% bromegrass hay or 60% hay and 40% corn and soybean hull supplements. Heifers consuming HAY or MID spent more *($P < 0.05$)* time at the bunk (avg, of 266 min/24 h) compared to heifers fed CORN or MIX (avg. of 237 min/24 h). However, the time spent in the bunk during the first 6 hours after feeding did not differ *($P > 0.05$)* among treatments, but as a percentage of the total time spent at the bunk during the first six hours, heifers fed MIX did spend more *($P < 0.05$)* time with their heads in the bunk (44.6%) than did heifers from the other treatments which were similar.

Implications

CORN supplementation (starch) linearly decreased diet NDF digestibility while MID supplementation (fiber) did not. These effects were found even with feeding higher quality hay (8.9% CP) compared to the lower quality hay (approximately 4% CP) used in experiments by Chase and Hibberd (1987) and Martin and Hibberd (1990). Heifers supplemented with CORN or MIX had *($P > 0.05$)* the lowest DM intake while heifers supplemented with MID had greater intakes. Interestingly, heifers supplemented with MIX consumed more DM during the first 6 h after feeding compared to the average of the other diets even though total minutes spent at the bunk were similar to the other treatments. This combination of starch and fiber (MIX) suggests a more aggressive feeding behavior.

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Table 1. Effects of increasing levels of cracked corn (CORN, Exp.1) or wheat middlings (MID, Exp.2) in a hay based diet on dry matter, nitrogen and NDF digestibilities when measured with wethers

	% CORN supplemented					% MID supplemented				
	0	5	10	20	SE	0	5	10	20	SE
DM intake, g	1360	1350	1360	1340		1330	1330	1330	1330	
NDF intake,g	848	812	775	702		753	740	726	700	
N intake, g	131	129	130	126		123	128	134	146	
DM dig.,%	54.8	60.7	56.9	59.8	1.74	53.8	60.0	55.9	59.5	1.76
NDF dig.,%[a]	53.7	57.5	47.3	47.5	2.22	43.2	47.5	42.8	44.9	2.11
N dig.,%[b]	53.3	59.3	50.8	55.7	2.04	62.4	67.8	66.2	70.9	1.56

[a] Linear response for NDF digestibility for Exp 1.

[b] Linear response for N digestibility for Exp.2

Table 2. Dry matter intake and feeding behavior of heifers fed hay only (HAY) or hay supplemented with corn (CORN), wheat middlings (MID), or a 50% mix of CORN and MID[c]

	HAY	CORN	MID	MIX	SE
DM intake, kg					
24 h	12.5[ab]	11.5[b]	12.9[a]	11.9[ab]	0.589
First 6 h,	6.1[b]	5.5[b]	5.7[b]	8.7[a]	0.593
First 6 h as % of 24 h	48.58[b]	45.46[bc]	40.51[c]	72.30[a]	3.60
Total time with head in the bunk, min/d	274.03[a]	241.82[b]	258.07[ab]	232.39[b]	14.46
Time during first 6 h at the bunk, min	97.66	84.14	97.24	101.97	10.36
First 6 h at bunk as a % of total time at the bunk	34.21[b]	33.93[b]	32.68[b]	44.61[a]	2.77

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

[c] Corn, MID and MIX fed at 20% of DM intake

Figure 1. Effects of increasing level of corn grain supplementation on NDF digestibility when wethers were fed grass hay-based diets

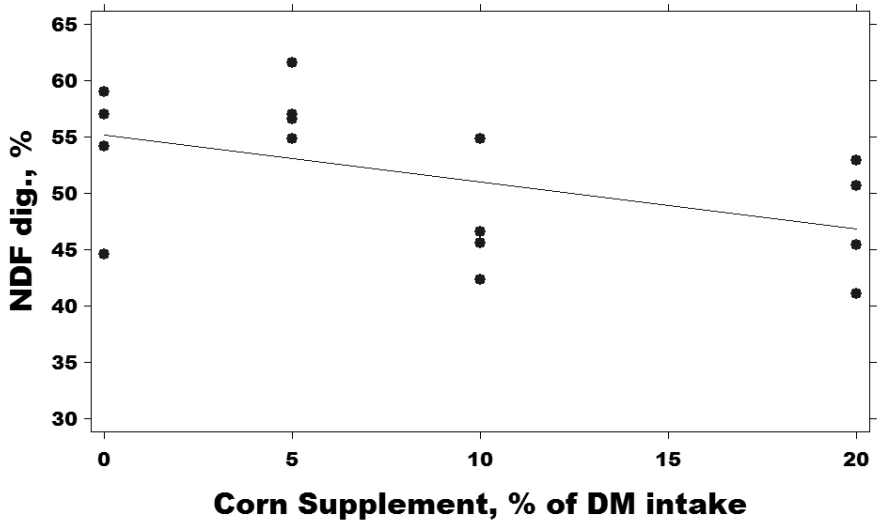
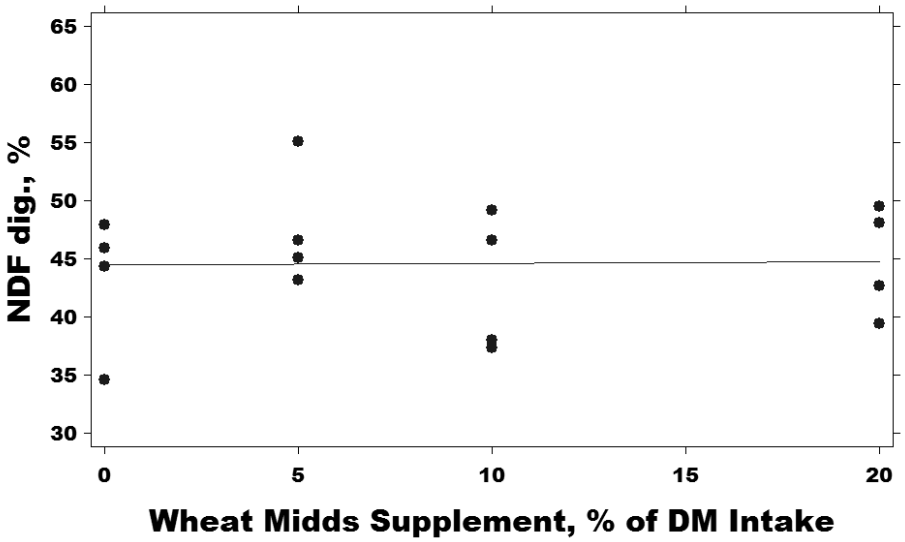


Figure 2. Effects of increasing level of wheat middlings supplementation on NDF digestibility when wethers were fed grass hay-based diets



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RUMINAL METABOLISM DURING CONTINUOUS CULTURE FERMENTATION WHEN REPLACING ALFALFA (*Medicago sativa* L.) HAY WITH BIRDSFOOT TREFOIL (*Lotus corniculatus* L.) HAY

C. M. Williams[1]*, C. M. Dschaak[1], J.-S. Eun[1], A. J. Young[1], and J. W. MacAdam[2]
[1]Department of Animal, Dairy, and Veterinary Sciences,
[2]Department of Plants, Soils, and Climate, Utah State University, Logan, UT

ABSTRACT: Three dual-flow fermentors (700 mL) were used to determine the effects of feeding birdsfoot trefoil (*Lotus corniculatus* L.) hay on microbial metabolism by mixed rumen cultures. We hypothesized that shifts in ruminal fermentation would occur when alfalfa (*Medicago sativa* L.) hay was replaced with birdsfoot trefoil (**BFT**) hay, resulting in improved N utilization and decreased methane production in vitro. Fermentors were incubated with filtered ruminal contents and allowed to adapt for 5 d to diets, followed by 3 d of sample collection. Three dietary treatments were assessed: 1) 100% alfalfa hay (**AH**), 2) 50% alfalfa hay + 50% BFT hay (**AHBFTH**), and 3) 100% BFT hay (**BFTH**). The experiment was conducted as a 3 × 3 Latin square design. Data were analyzed using the MIXED procedure of SAS. Culture pH averaged 6.44, and feeding the AH or the AHBFTH resulted in a higher culture pH compared with the BFTH (P = 0.01). Methane production based on its concentration in fermentor headspace was not influenced by treatments. However, ammonia-N concentration decreased in cultures offered the AHBFTH compared with the AH, and it decreased further in cultures receiving the BFTH compared with the AH and the AHBFTH (P < 0.01). Total volatile fatty acid production averaged 32.9 mmol/d and was not affected by treatments. Molar proportions of acetate and propionate and acetate to propionate ratio were not influenced by treatments. Our data indicate that feeding BFT altered the metabolic pathways of in vitro ruminal fermentation with beneficial modification of N use by mixed cultures of ruminal microorganisms, whereas cultures fed BFT did not have any negative impact on ruminal fermentation. Decreased ammonia-N concentration by feeding BFT as hay may make it suitable as an effective source of forage to increase the passage of microbial protein and improve forage N utilization by ruminants.

Key words: alfalfa hay, ammonia-N, birdsfoot trefoil hay, continuous cultures

Introduction

High quality alfalfa (*Medicago sativa* L.) is palatable and often maximizes intake and production of ruminants. High quality alfalfa is low in fiber and high in protein compared to other forages, which makes it an excellent complement for grains and other forages in ruminant rations. However, the full benefit of alfalfa protein is not realized due to its poor utilization by the animal; ruminal microbes degrade alfalfa protein too rapidly, resulting in excessive excretion of nitrogenous waste by the animal.

Condensed tannins (**CT**), the polymers of flavanol units, are probably the most extensively studied plant secondary metabolites with reference to their physiological and nutritional consequences, and are the most common type of tannins found in forage legumes, trees, and shrubs (Barry and McNabb, 1999). Moderate levels of CT (2 to 4% on DM basis) bind to protein by hydrogen bonding at near neutral pH (pH 6.0 to 7.0) in the rumen to form CT-protein complexes, but dissociate and release bound protein at pH less than 3.5 in the abomasum (Barry et al., 2001). Thus, CT-containing forages can protect dietary protein against degradation in the rumen and increase amino acid supply to the abomasum and small intestine, resulting in improved nutritional status of the animal. In addition, many studies have reported that feeding CT-containing forages to ruminants may reduce methane (**CH_4**) emissions (Woodward et al., 2004; Puchala et al., 2005), although the reduction in CH_4 is confounded with changes in forage quality, and the exact mechanism is not clear (Animut et al., 2008).

Birdsfoot trefoil (*Lotus corniculatus* L.) is a widely distributed legume that contains a moderate concentration of CT (3 to 4% of DM). In North America, alfalfa is grown in preference to birdsfoot trefoil (**BFT**) on deep, well-drained soils, while BFT is better adapted to soils too acidic or limited in fertility, texture, or rooting depth for successful alfalfa production (MacAdam et al., 2006). However, BFT is persistent and high-yielding in multiple year trials in the climate and soils of the Intermountain West (MacAdam and Griggs, 2006). With new knowledge of CT biosynthesis, it may be possible to engineer alfalfa to produce CT that provide protein protection in the rumen (Pang et al., 2007). Where BFT is persistent and high-yielding, it provides a non-engineered alternative to improve N utilization and reduce excretion of nitrogenous waste and enteric CH_4 emission by ruminants.

Using continuous culture fermentation, the objective of the current study was to evaluate the effects of feeding BFT on ruminal fermentation and digestibility in continuous cultures. We hypothesized that replacing alfalfa hay with BFT hay would result in improved N utilization and decreased CH_4 production.

Materials and Methods

Forage harvest, diets, and experimental design. Birdsfoot trefoil cvs. 'Grasslands Goldie' and alfalfa cv. WL326GZ were grown under irrigation at the Greenville Research Farm in North Logan, Utah. Forage was harvested June 17, July 30, and September 10, 2008 and immediately dried at 60°C for two days. Prior to use in the fermentors, the forage was dried at 55°C for 48 h and ground through a 4.0-mm screen (standard model 4, Arthur Thomas Co., Philadelphia, PA).

Three dietary treatments were compared in a 3 × 3 Latin square design with three periods as repeated runs. Treatments consisted of: 1) 100% alfalfa hay (**AH**), 2) 50% AH + 50% BFT hay (**AHBFTH**; DM basis), and 3) 100% BFT hay (**BFTH**).

Continuous culture conditions. A three-unit dual flow continuous culture system similar to that described by Teather and Sauer (1988) was used with inoculums obtained 4 h after the morning feeding (1100 h) from two ruminally cannulated dairy cows fed a TMR formulated to meet the nutrient requirements of dairy cows in early lactation. Ruminal contents were obtained from various locations within the rumen and immediately transferred in a sealed flask to the laboratory where the ruminal contents were strained through polyester material (PeCAP, pore size 355 µm; B & S H Thompson, Ville Mont-Royal, Quebec, Canada) under a stream of oxygen-free CO_2. The strained ruminal fluid was dispensed (approximately 700 mL) into the culture fermentor vessels that had been warmed to 39°C and flushed with oxygen-free CO_2. To displace O_2 and maintain anaerobic conditions in the vessels, the rate of CO_2 was fixed at 20 mL/min throughout the experiments. A circulating water bath was used to maintain the temperature of the fermentors at 39°C. Continuous stirring of the fermentor contents was achieved with the aid of a central paddle set at a speed of 25 rpm (Eun et al., 2004). The liquid dilution rate of the cultures was maintained at 10%/h by regulating the addition of artificial saliva prepared as described by Slyter et al. (1966). Alfalfa hay pellets (20 g DM basis) were added to the fermentors on d 1, followed by gradually increased experimental diets and decreased alfalfa hay pellets on subsequent days during the adaptation period. On d 5, all three fermentors were receiving 100% experimental diet, with samples being taken on d 6 to 8. Two equal portions of the experimental diets totaling 20 g (DM basis) were placed in each fermentor daily at 0800 and 2000 h.

Table 1. Nutrient concentration of dietary treatments

Item	Dietary treatment[1]		
	AH	AHBFTH	BFTH
DM, %	94.7	94.1	93.7
OM, % of DM	92.0	91.1	90.3
CP, % of DM	20.7	19.7	16.8
NDF, % of DM	43.8	39.0	38.8
ADF, % of DM	35.8	30.9	31.9
CT[2], % of DM	0.35	ND[3]	2.08

[1]AH = 100% alfalfa hay; AHBFTH = 50% AH and 50% birdsfoot trefoil hay (DM basis); BFTH = 100% birdsfoot trefoil hay.

[2]CT = condensed tannins.

[3]ND = not determined.

Sample Collection and Chemical Analysis. Culture pH readings were taken every hour for 12 h using a pH meter on d 6 and 7. Ten microliters of headspace gas samples from the fermentor were drawn into a gastight syringe (Hamilton Co., Reno, NV) at 0, 3, 6, 9 and 12 h after feeding and analyzed for methane using a GLC (model CP-3900; Varian, Walnut Creek, CA). At the same time, 5-mL of thoroughly mixed culture contents was collected and added to 1 mL of 25% meta-phosphoric acid for VFA analysis. Separate 5-mL samples of the mixed culture contents was added to 1 mL of 1% sulfuric acid and samples were retained for ammonia-N (NH_3N) determination. The VFA were quantified using a GLC (model 6890 series II; Hewlett Packard Co., Avandale, PA) with a capillary column (30 m ×0.32 mm i.d., 1 µm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium. Concentration of NH_3-N in the ruminal contents was determined as described by Rhine et al. (1998), using a MRX plate reader (Dynex Technologies, Chantilly, VA).

Effluent samples were also taken every 24 h on d 6 and 7 for digestibility determination. At the termination of each run on d 8, ruminal contents were harvested to assess microbial protein and ruminal lipid profiles.

The dietary treatments and effluent samples were analyzed for DM (method 930.15) and N (method 990.03) according to AOAC (1999). The NDF and ADF, both inclusive of residual ash, were determined according to Hall et al. (1998) with the method modified for use with the Ankom^{200} Fiber Analyzer (Ankom Technology, Macedon, NY). Heat stable α-amylase and sodium sulfite were used in the NDF analysis. Condensed tannins were determined using the method of Terrill et al. (1992).

Statistical analysis. All the data in this study were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) with a repeated measures treatment structure. The model included treatment as a fixed effect with period and fermentor as random effects. The Kenward-Roger option was used to estimate denominator degrees of freedom. Variables that were repeated in time were analyzed using the same mixed model but with sampling time added to the model, and a repeated statement included. The covariance structure that resulted in the lowest values for the Akaike's information criteria and Schwartz's Bayesian criterion was used (Littell et al., 1998) for variables with repeated measures over time. Effects of the factors were declared significant if $P < 0.05$ and trends were accepted if $0.05 < P < 0.10$.

Results

The BFTH contained four and five percentage units less CP and NDF, respectively compared to the AH (Table 1). Culture pH decreased in the fermentor offered the BFTH compared to those received the AH or the AHBFTH (Table 2). Methane production based on its concentration in fermentor headspace was not influenced by treatments. However, NH_3N concentration decreased in cultures offered the AHBFTH compared with the AH, and it decreased further in cultures receiving the BFTH compared with the AH and the AHBFTH ($P < 0.01$). Total VFA production averaged 32.9 mmol/d and was not affected by treatments. Molar proportions of acetate, propionate, butyrate, and acetate to propionate ratio were not influenced by treatments.

Table 2. Effect of diets replacing alfalfa hay with birdsfoot trefoil hay on ruminal fermentation characteristics in mixed cultures of ruminal microorganisms

Item[1]	Dietary treatment[2]			SE	*P*
	AH	AHBFTH	BFTH		
Culture pH	6.45[a]	6.48[a]	6.40[b]	0.036	0.01
CH_4	9.18	8.57	9.40	0.058	0.58
NH_3N	22.7[a]	19.3[b]	16.4[c]	1.77	0.01
TVFA	35.6	30.3	32.9	4.71	0.59
IVFA					
A	66.6	66.8	66.2	3.20	0.82
P	23.3	24.5	24.3	0.89	0.28
B	4.42	3.83	5.21	1.812	0.32
A:P	2.87	2.75	2.74	0.216	0.44

[1]CH_4 = methane, mmol/d; NH_3N = ammonia-N, mg/dL; TVFA = total VFA, mM; IVFA = individual VFA, mol/100 mol; A = acetate; P = propionate; B = butyrate.

[2]AH = 100% alfalfa hay; BFT = 100% birdsfoot trefoil; AHBFT = 50% AH + 50% BFT (DM basis).

[a,b,c]Means with different superscript differ at $P < 0.05$.

Table 3. Effect of diets replacing alfalfa hay with birdsfoot trefoil hay on fermentability, digestibility, and microbial N production in mixed cultures of ruminal microorganisms

Item[1]	Dietary treatment[2]			SE	*P*
	AH	AHBFTH	BFTH		
FER, %	25.9	23.7	25.5	3.32	0.90
NDFD, %	47.2	46.2	44.7	1.98	0.51
MN flow	0.38	0.37	0.48	0.103	0.53
NH_3N flow	0.38[a]	0.32[b]	0.28[c]	0.029	0.01
EMPS	22.7	25.1	24.0	4.74	0.77

[1]FER = fermentability = ((substrate used for VFA, CO_2 + CH_4 + 2 H_2O, and microbial biomass) ÷ (DM fed)) × 100; NDFD = NDF digestibility; MN flow = microbial N flow, g/d; NH_3N flow = ammonia-N flow, g/d; EMPS = efficiency of microbial protein synthesis (g of N/kg of OM digested).

[2]AH = 100% alfalfa hay; BFT = 100% birdsfoot trefoil; AHBFT = 50% AH + 50% BFT (DM basis).

[a,b,c]Means with different superscript differ at $P < 0.05$.

Dietary treatments did not affect fermentability (Table 3). Digestibility of NDF averaged 46%, and it was not influced by dietary treatment. Although feeding the BFTH increased microbial N flow compared to the AH, there was no treatment effect on microbial yield. Replacing the AH with the BFTH decreased NH_3N flow. Efficiency of microbial protein synthesis averaged 23.9 g of N/kg of OM digested across dietary treatments and was not influenced by treatments.

Discussion

Feeding the BFTH to mixed cultures failed to reduce CH_4 production in our study. Various other studies have indicated that feeding fresh CT-containing forages decreased CH_4 emissions in vivo and in vitro (Woodward et al., 2004; Puchala et al., 2005; Tavendale et al., 2005a,b; Animut et al., 2008). Using in vitro batch incubations, Tavendale et al. (2005a) reported that the accumulated CH_4 volume at 12 h for big trefoil (*Lotus pedunculatus*) was lower (8.8 mL) than alfalfa (12.5 mL). In another study, Tavendale et al. (2005b) showed that BFT was intermediate in CH_4 production among various CT-containing forages, and CT concentration in forage DM was inversely related to CH_4 production. Purified tannin extracts from tannin-containing plants exhibited a range of antimicrobial activity (Min et al., 2008), suggesting that the source of CT and their chemical compositions may influence antimicrobial activity to methanogens. More research is needed in this area to make any significant conclusions.

In vitro and in vivo trials have consistently demonstrated a reduction in proteolysis as a consequence of dietary CT (Waghorn, 2008). Barry and McNabb (1999) reported reduced ammonia flow from the rumen for sheep fed fresh BFT, but this is the first report of reduced ammonia flow for ruminants fed BFT hay. The remarkable reduction of NH_3N concentration and flow with the BFTH observed in the current study supports primary function of the dietary CT by forming CT-protein complexes, resulting in reduced ruminal protein degradation. Agriculture production has been identified as a primary contributor of atmospheric reactive N in the form of ammonia and ammonium (Faulkner and Shaw, 2008). Therefore, feeding BFT as a main forage source to ruminants would be a sound practice to improve environmental performance. The reduced NH_3N concentration did not contribute to improving efficiency of microbial protein synthesis and microbial yield in this study. Given the diet composition with 100% hay used in this study, however, microbes in cultures must need readily available energy to capture forage N into microbial protein. Hence feeding BFT to high producing dairy cows and rapid growing beef cattle would improve ruminal microbial yield, because TMR diets to the animals typically contain high level of readily available energy from grains.

Implications

Sizable reduction of NH_3N flow by feeding the BFTH found in this study promises beneficial impact of N utilization by ruminants. However, it is recommended that the positive effects of BFT be further evaluated in in vitro and in vivo feeding studies using TMR diets based on BFT

as a main forage. Birdsfoot trefoil has the potential to not only improve both the economic and environmental sustainability of grazing-based dairy operations, but could become a value-added hay product marketed to confinement dairies wishing to improve N use efficiency and reduce the N content of urine.

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GENETIC PARAMETERS FOR PERCENT INTRAMUSCULAR FAT, MARBLING SCORE, SCROTAL CIRCUMFERENCE, AND HEIFER PREGNANCY IN RED ANGUS CATTLE

C. M. McAllister, S. E. Speidel, B. W. Brigham, D. H. Crews, Jr., and R. M. Enns

Department of Animal Sciences, Colorado State University, Fort Collins

ABSTRACT: Selection criteria for yearling bulls commonly include indicators of fertility and carcass merit, such as scrotal circumference (**SC**) and intramuscular fat percentage (**IMF).** Genetic correlation estimates between ultrasound traits such as IMF and carcass marbling score (**MS**) with fertility traits SC and heifer pregnancy (**HP**) have not been reported. Therefore the objective of this study was to estimate the genetic parameters among the indicator traits IMF and SC, and the economically relevant traits MS and HP. Records for IMF (n = 73,051), MS (n = 15,260), SC (n = 43,487), and HP (n = 37,802) were obtained from the Red Angus Association of America, and a 4-generation ancestral pedigree (n = 10,460) was constructed from the 8,915 sires represented in the data. (Co)Variance components were estimated using a multivariate sire model and average information REML procedures to obtain estimates of heritability and genetic correlations. Fixed effects included contemporary group and the linear effect of age at measurement for all traits, and an additional effect of age of dam for both HP and SC. The random effect of sire was included to estimate additive genetic effects, which were assumed to be continuous for IMF, MS, and SC but a probit threshold link function was fitted for HP. Generally moderate heritability estimates of 0.29 ± 0.01, 0.35 ± 0.06, 0.32 ± 0.02, and 0.17 ± 0.01 were obtained for IMF, MS, SC, and HP on the underlying scale, respectively. The confidence interval for the estimated genetic correlation between MS and HP (0.10 ± 0.15) included zero, suggesting negligible genetic associations. The genetic correlation between MS and IMF was high (0.80 ± 0.05) but the estimate for HP and SC (0.05 ± 0.09) was near zero, as were the estimated genetic correlations of SC with MS (0.01 ± 0.08) and IMF (0.05 ± 0.06), and for HP with IMF (0.13 ± 0.09). These results suggest that concomitant selection for increased fertility and carcass merit would not be antagonistic.

Key words: genetic parameters, heifer pregnancy, marbling score, percent intramuscular fat, scrotal circumference.

Introduction

Reproductive traits have been shown to have a large impact on profitability when compared to other economically relevant traits (Golden et al., 2000), such as growth performance and carcass merit, at the cow calf level (Melton, 1995). Economic incentives are currently given for improved carcass merit to producers who retain ownership and market on various grid programs. These economic signals indicate a need for concomitant selection for improved carcass merit and fertility for commercial producers to improve total revenue.

Yearling bulls from the seedstock segment of the beef industry are commonly performance tested prior to sale. These tests rank bulls based on phenotypic performance for average daily gain, ultrasound indicators of carcass merit, and indicators of fertility such as yearling scrotal circumference (**SC).** Bull buyer selection criteria for yearling bulls commonly include these indicators of fertility (Martinez-Velazquez et al., 2003; Toelle and Robison, 1985; Brinks et al., 1978) and carcass merit (Crews and Kemp, 2002), such as SC and intramuscular fat percentage (**IMF**).

Optimal selection decisions based on multiple traits must consider genetic antagonisms among the traits of interest. Genetic correlation estimates for carcass merit traits such as ultrasound IMF and carcass marbling score (**MS**) with fertility traits such as SC and heifer pregnancy (**HP**) have not been reported. Therefore, the objective of this study was to investigate potential antagonisms between fertility and carcass merit by estimating genetic parameters among IMF, SC, MS and HP in Red Angus cattle.

Materials and Methods

Because data were obtained from the Red Angus Association of America (**RAAA**) databases, Animal Care and Use Committee approval was not obtained for this study. Records for IMF, MS, SC, and HP were obtained from the RAAA along with associated pedigree information on animals born between 1977 and 2007. The RAAA defines heifer pregnancy as a heifer that has an observable calving observation at 2 years of age, falling within a calving interval designated by a producer-defined breeding season length. A successful heifer pregnancy is denoted as a "1" and a failure as a "0".

Contemporary groups (CG) for IMF, MS, and SC were defined as combinations of yearling management code, yearling working group, sex, and measurement date whereas CG for HP additionally included heifer management code. Data were edited to remove records for animals that had observations 5 standard deviations or greater from their respective CG means, competed in CG of less than 5 animals, and CG with no variation. These edits resulted in a total of 149,478 animals in the final data set with numbers of records for IMF, MS, SC, and HP of 73,051, 15,260 43,487, and 37,802 and unique CG of 4,865, 442, 1,813, and 1,670, respectively. For the

purposes of variance component estimation, a 4-generation ancestral sire pedigree (n = 10,460) was constructed from the 8,915 unique sires represented in the final dataset.

A multivariate sire model was used to estimate direct genetic and residual (co)variance parameters. Fixed effects included contemporary group and the linear effect of age at measurement (d) for all traits. Age at measurement for HP was defined as the age at which the heifer was initially exposed to breeding. An additional effect of age of dam (2-, 3-, 4-, 5- to 9-, $\geq$ 10-yr of age) was fitted for both HP and SC (BIF, 2002). The random effect of sire was included to estimate additive genetic effects, which were assumed to be continuous for IMF, MS, and SC. Due to the binary nature of HP observations, a probit threshold link function was fitted for HP (Gianola and Foulley, 1983; Harville and Mee, 1984). The linear model used can be described in matrix notation as

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \end{bmatrix} = \begin{bmatrix} X_1 & 0 & 0 & 0 \\ 0 & X_2 & 0 & 0 \\ 0 & 0 & X_3 & 0 \\ 0 & 0 & 0 & X_4 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \end{bmatrix}$$

$$+ \begin{bmatrix} Z_1 & 0 & 0 & 0 \\ 0 & Z_2 & 0 & 0 \\ 0 & 0 & Z_3 & 0 \\ 0 & 0 & 0 & Z_4 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \\ u_3 \\ u_4 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \end{bmatrix},$$

where known incidence matrices $\mathbf{X_i}$ and $\mathbf{Z_i}$ relate unknown fixed ($\mathbf{b_i}$), and direct genetic ($\mathbf{u_i}$) effects, respectively, to observations in $\mathbf{y_i}$ with subscripts 1, 2, 3, and 4 denoting IMF, MS, SC, and pseudo observations for HP on the underlying scale, respectively, and $\mathbf{e_i}$ is a random residual term specific to animals with records for trait i.

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

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

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

where **u** and **e** are vectors of additive direct genetic and residual variance, respectively for each trait i. **A** is the Wright's numerator relationship matrix, $\otimes$ is the Kronecker product operator, $\mathbf{G_0}$ is the additive genetic (co)variance matrix and $\mathbf{R_n}$ is a matrix of residuals such that with only trait 1, trait 2, trait 3, or trait 4 measured, $\sigma^2_{e_1}, \sigma^2_{e_2}, \sigma^2_{e_3}, \sigma^2_{e_4}$ will be on the diagonal with subscripts defined above. With 2 traits measured, $\sigma^2_{e_i}$ will be on the diagonal and σ_{e_i,e_j} will be on the corresponding off-diagonal, where $\sigma^2_{e_i}$ is the variance due to residual effects for trait i, and σ_{e_i,e_j} is the residual covariance for i^{th} and j^{th} traits measured on the same animal with $i \neq j$. Due to the nature of the traits analyzed, all traits could not be measured on the same animal, and therefore most residual covariances were by definition zero. For instance, the sex limited expression of fertility traits SC and HP could not be measured on the same animal, and breeding animals would not have MS records. The genetic parameters for all traits and their standard errors were estimated using ASREML (Ver. 2.0, VSN International, Ltd., Hemel Hempstead, UK) which employs an average information REML algorithm.

Results and Discussion

Summary statistics for phenotypic measures of IMF, MS, SC, and HP are presented in Table 1.

Table 1. Summary statistics[1] for ultrasound intramuscular fat (IMF, %), carcass marbling score (MS), yearling scrotal circumference (SC, cm), and heifer pregnancy (HP) in Red Angus.

	N	Mean	SD	Min[1]	Max[1]
IMF	73,051	3.79	1.03	0.77	10.05
MS	15,250	5.42	1.00	0.30	10.50
SC	43,487	35.24	2.70	20.50	48.00
HP	37,802	0.80	0.40	0.00	1.00

[1] Min = Minimum; Max = Maximum

Mean IMF (3.79) and MS (5.42) in this data were similar to previous reports of Simmental (Crews et al., 2003) and Angus data (MacNeil and Northcutt, 2008). The SC summary statistics were equivalent to the report of Crews and Enns (2008) using data from RAAA. The RAAA does not produce a SC EPD, therefore large phenotypic differences from year to year are not expected do to the lack of selection pressure. The average HP rate in these data was high with an 80% success rate.

Shown below in Table 2 are the estimates of genetic (co)variances and their corresponding parameters for all traits analyzed. Phenotypic variances as well as residual correlations are shown below in Table 3. Generally moderate heritability estimates of 0.29 ± 0.01, 0.35 ± 0.06, 0.32 ± 0.02, and 0.17 ± 0.01 were obtained for IMF, MS, SC, and HP on the underlying scale, respectively. The current heritability estimate for IMF was lower than the 0.47 ± 0.02 estimate of Speidel et al. (2007) for Red Angus cattle. The MS heritability estimate was similar to the previous sire model estimate (0.26 ± 0.04) reported by Wilson et al., (1993) for Angus cattle, but less than animal model estimates of MacNeil and Northcutt, (2008) and Crews et al. (2003) who used field data from Angus and American Simmental, respectively. The heritability estimate for SC was lower than previous studies reporting SC to be highly heritable ($h^2 > 0.5$) (Evans et al., 1999; Crews and Enns, 2008). The current HP heritability estimate (0.17 ± 0.01) is similar to a previous estimate reported by Evans et al. (1999) supporting that heifer pregnancy is heritable on the underlying scale and genetic progress can be made over time given adequate breeding value accuracy and selection intensity.

The genetic correlation between MS and IMF was high (0.80 ± 0.05) and similar to previous estimates of 0.69 to 0.74 for Simmental heifers and bulls, respectively, (Crews et al., 2003) and the 0.52, 0.66, and 0.84 estimated on heifers, bulls, and steers, respectively, as separate traits (MacNeil et al., 2008). Robertson (1959) stated that estimates of genetic correlation $\geq$ 0.8 indicate traits are genetically equivalent. Therefore these results support previous research suggesting that genetic evaluation of MS is enhanced by inclusion of IMF as an indicator trait measured on breeding animals.

The current genetic correlation estimate between HP and SC (0.05 ± 0.09) was near zero. These results are in agreement with Evans et al. (1999) who estimated a corresponding genetic correlation of 0.002. Favorable additive genetic relationships of SC and age at puberty have been reported in the past (Toelle and Robison, 1985; Brinks et al., 1978). Yet, genetic relationships between age at puberty and heifer pregnancy rates has yielded conflicting results (Moser et al., 1996). Current correlation estimates between direct genetic effects on HP and SC suggest that development and use of a HP EPD would be more efficient for increasing the proportion of females calving at 2 yrs of age compared to selection criteria based on yearling SC measurements.

The confidence interval for the estimated genetic correlation between MS and HP (0.10 ± 0.15) was large, suggesting a negligible genetic association. Similarly, the estimated genetic correlations of SC with MS (0.01 ± 0.08) and IMF (0.05 ± 0.06), and for HP with IMF (0.13 ± 0.09) all were close to zero. These results indicate that genetic merit for fat deposition at approximately one year of age has little genetic association with fertility measures.

Implications

Analysis of the indicator traits IMF and SC with the economically relevant traits MS and HP suggest that concomitant selection for increased fertility and carcass merit would not be antagonistic. Based on their high genetic correlation, IMF is an adequate selection criterion for improved MS, and would increase accuracy of MS estimated breeding values. However the low direct genetic correlation between SC and HP indicates that genetic progress can be made more efficiently by selection on HP EPD rather than the indicator SC.

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Table 2. Estimates of heritability ($h^2 \pm SE$) for all traits[1] (on the diagonal, indicated by boldface), genetic covariances among traits (above the diagonal), and genetic correlations ($r_g \pm SE$) derived from them (below the diagonal)

	IMF	MS	SC	HP
IMF	**0.29 ± 0.01**	0.198	0.030	0.025
MS	0.801 ± 0.055	**0.35 ± 0.06**	0.0115	0.019
SC	0.048 ±0.058	0.014 ± 0.076	**0.32 ± 0.02**	0.0337
HP	0.126 ± 0.089	0.096 ± 0.150	0.054 ± 0.091	**0.17 ± 0.01**

[1]IMF = Intramuscular Fat Percentage (%); MS = Marbling Score; SC = Scrotal Circumference (cm); HP = Heifer Pregnancy

Table 3. Estimates of phenotypic variance for all traits[1] (on the diagonal, indicated by boldface), residual covariances among traits (above the diagonal), and residual correlations ($r_e \pm SE$) derived from them (below the diagonal)

	IMF	MS	SC	HP
IMF	**0.291**	0	0.022	0.011
MS	0	**0.349**	0	0
SC	0.048 ± 0.058	0	**0.317**	0
HP	0.016 ± 0.009	0	0	**0.166**

[1]IMF = intramuscular fat percentage (%); MS = marbling score; SC = scrotal circumference (cm); HP = heifer pregnancy

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MICROSATELLITE ETH10 IN THE PROMOTER OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION-6 GENE PREDICTS 205-d WEIGHT IN RED ANGUS CATTLE

K. L. DeAtley[1], G. Rincon[2], C. R. Farber[2], J. F. Medrano[2], R. M. Enns[3], D. VanLeeuwen[4], G. A. Silver[1], M. G. Thomas[1]

[1]Department of Animal and Range Science; New Mexico State University, Las Cruces 88003; [2]Department of Animal Science; University of California, Davis 95616; [3]Department of Animal Science, Colorado State University, Fort Collins 80523; [4]Agriculture Biometric Service, New Mexico State University, Las Cruces 88003

ABSTRACT: ETH10 is a GT microsatellite within the promoter of signal transducer and activator of transcription-6 (STAT6) gene on bovine chromosome 5. This protein is involved in the cell signaling cascade subsequent to hormones such as leptin and GH binding their receptors. ETH10 has been included in several studies searching for QTL associated with growth and body composition. This polymorphism is also included in the panel of genetic markers recommended by the International Society of Animal Genetics for DNA-based parentage testing in cattle. Red Angus Association of America requires these tests for AI sires and embryo donor dams. Allelic size of this polymorphism is based upon standardized PCR amplicons that range from 199 to 225 bp in size, which is dependent on the number of GT repeats within the gene sequence. We investigated association of ETH10 genotypes with growth and ultrasound carcass phenotypes of cattle registered with Red Angus Association of America (n = 5,058 from 1966 to 2007). Associations of genotypes to phenotypes were evaluated with a mixed effects model including year of birth, sex of the animal, date of birth, genotype, and breeding method as fixed effects and sire as a random source of variation. Nine alleles were detected; however, only alleles 215, 217, 219, 221, and 223 had minor allele frequency ≥ 5%. All genotypic frequencies ≥ 5% included allele 217; therefore, genotypes 215/217, 217/217, 217/219, 217/221, and 217/223 were used in analyses. None of these genotypes deviated from Hardy-Weinberg Equilibrium. Cattle with 217/219 genotype tended to have heavier (P = 0.07) adjusted 205-d weight than cattle of the 215/217, 217/217, and 217/221 genotypes (271.43 > 264.08, 264.95, and 266.37 ± 2.98 kg). ETH10 genotypes appear to be associated with the phenotype of 205-d weight in red Angus cattle. Even though this study does not allow determination of linkage disequilibrium on chromosome 5, results provide support for additional investigations involving STAT6 as a positional candidate gene in studies of animal growth.

Key words: Cattle, genotype, growth, Red Angus

Introduction

Initial genome mapping efforts and association studies of genotype to phenotype used microsatellites, even though today most efforts involve SNP. Microsatellites have been identified in both coding and non-coding regions of the genome (Sellner et al., 2007). Farber and Medrano (2003) reported that ETH10, a microsatellite included in the International Society of Animal Genetics parentage panel, was a GT repeat located in the promoter region of the signal transducer and activator of transcription-6 (STAT6) gene on bovine chromosome 5. This protein is involved in the cell signaling cascade subsequent to hormones such as leptin and GH binding their receptors.

Genotypes of ETH10 were used to detect QTL for growth traits in Angus x Brahman crossed cattle (Kim et al., 2003) and strongly associated with phenotypes of marbling in Wagyu cattle (Barendse, 2002). Previously, we reported that genotypes of ETH10 were also associated with traits of birth weight, percent fat within LM, and LM per BW in Brangus cattle (i.e., 3/8 Brahman x 5/8 Angus; DeAtley et al., 2008). These genotype and phenotype data were obtained from the International Brangus Breeders Association as they require parentage testing of all AI sires and embryo transfer donor dams.

The Red Angus Association of America also requires parentage testing for cattle used in artificial breeding programs using the International Society of Animal Genetics DNA marker panel which includes ETH10. The objective of this study was to conduct association analyses utilizing ETH10 genotypes and growth and ultrasound carcass phenotypes of cattle registered with the Red Angus Association of America.

Materials and Methods

Genotyping was performed at MMI Genomics Inc. a subsidiary of MetaMorphix Inc. (Davis, CA) at the request of the Red Angus Association of America. Genotype and phenotype data were queried from the association's database (n = 5,058 from 1966 to 2007).

Data were analyzed with SAS (Ver. 9.1.3 SAS Inst. Inc., Cary, NC), which included genetic analysis tools (Saxton et al., 2004). Frequencies of alleles and genotypes

were determined using Proc Allele, which also outputted tests of Hardy-Weinberg equilibrium. If frequency of genotype category was greater than 5%, then data were considered appropriate for use in mixed effects analyses. These mixed model associations of genotype to phenotype were conducted with Proc Mixed. The model was:

$y_{ijklm} = \mu + A_i + B_j + C_k + D_l + E_m + e_{ijklm}$ where

y_{ijklm} = phenotypic value of trait,

μ = population mean,

A_i = fixed effect of genotype (i.e., 215/217, 217/217, 217/219, 217/221, or 217/223),

B_j = fixed effect of sex (i.e., male or female),

C_k = fixed effect of breeding method (i.e., AI, ET = embryo transfer, NS = natural service),

D_l = fixed effect of year of birth (1966 to 2007),

E_m = random effect of sire (i.e., mean = zero, variance = σ_s^2; Z statistic used to test if Ho: $\sigma_s^2 = 0$, and

e_{ijklm} = random residual error.

Contemporary group and breeder were other logical sources of information in these data; however, they were confounded with year of birth due to the data structure, therefore, eliminated from the model.

Traits analyzed with this model were birth weight, 205-d weight and 365-d weight, ADG, LM area, thickness over the 12th and 13th rib, % intra-muscular fat of LM, rump fat, and LM area per kilogram of BW. Weights were adjusted using procedures of the Beef Improvement Federation (2006). Age of dam information was not available due to data structure; therefore, birth weight and 205-d weight were only adjusted for age. Carcass measures were collected with ultrasound and adjusted to 365-d of age by the Red Angus Association of America.

Breeding method (C_k) and individuals born by embryo transfer procedures were not included in analyses of the maternal traits of birth weight and 205-d weight. The interaction of genotype and year of birth was also evaluated in preliminary testing of models, but was omitted due to lack of significance. If genotype terms were found to be important ($P < 0.1$), preplanned pair wise comparisons of least squares means were generated with PDIFF. These mean separation tests were executed using LSMEANS of the mixed procedure, which included Bonferroni's adjustment.

Results and Discussion

Allelic and Genotypic Frequencies. ETH10 is a microsatellite in the International Society of Animal Genetics parentage panel. Allelic size of this polymorphism is based upon PCR primer amplicons that range from 199 to 225 bp in size, which is dependent on the number of GT repeats within the gene sequence. In the current study, nine alleles and 21 genotypes were observed (Table 1). Allelic and genotypic frequencies appeared comparable to other *Bos taurus* populations (Bicalho et al., 2006).

The genotype term (A_i; i.e., 215/217, 217/217, 217/219, 217/221, and 217/223) was derived from 2 evaluations of allele and genotype frequecies. The first evaluation revealed that the 215, 217, 219, 221, and 223 alleles were most prevalent in the population. The second evaluation revealed that all genotypic frequencies $\geq$ 5% included the 217 allele; therefore, genotypes utilized in analysis were 215/217, 217/217, 217/219, 217/221, and 217/223, respectively. Genotypes appeared to be in Hardy-Weinberg equilibrium ($X^2 = 0.9908$; $P > 0.73$), so deemed useful for genotype to phenotype association analyses.

Association of Genotype to Phenotype. Kim et al. (2003) reported that genotypes of ETH10 were useful in detecting QTL associated with birth weight and 365-d weight in Brahman x Angus cattle. These analyses were on a whole genome basis. Development of the bovine genome map provided capability to visualize candidate gene(s) to investigate their relevance in genotype to phenotype association studies. Specifically, the STAT6 gene is located in a 23 mega-base region on bovine chromosome five that composes QTL associated with growth and body composition (Rincon et al., 2007). ETH10 is located within the promoter of the STAT6 gene, which is a protein involved in the cell signaling cascade subsequent to hormones such as leptin and GH binding their receptors (Farber and Medrano, 2003; Rawlings et al., 2004). Even though this study does not allow determination of linkage disequilibrium on chromosome 5, results provide additional support for investigations involving STAT6 as a positional candidate gene.

In the current study, sex, breeding method and year of birth were significant factors ($P < 0.05$), and the variance due to sires differed from zero ($P < 0.05$) in 87.5% of the traits analyzed. Red Angus cattle with 217/219 genotype tended to have heavier ($P = 0.07$) 205-d weight than cattle of the 215/217, 217/217, and 217/221 genotypes (Table 2). Associations of ETH10 genotypes have also been reported for traits of marbling in Asian breeds of cattle (Smith et al., 2001; Barendse, 2002) and birth weight, percent fat within LM, and LM per body weight in Brangus cattle (DeAtley et al., 2008).

There currently is no evidence to suggest that ETH10 influences gene function; however, the results of this study and the QTL findings of Kim et al. (2003) suggest that the STAT6 gene could contain quantitative trait nucleotides (QTN; i.e., functional mutations) that are linked with ETH10 on bovine chromosome 5. Cumulatively, results provide support for additional investigations involving STAT6 as a positional candidate gene in studies of animal growth.

Implications

ETH10, a microsatellite included in the International Society of Animal Genetics parentage panel, is located in the promoter region of the signal transducer and activator of transcription-6 gene on bovine chromosome 5. This protein is involved in the responsiveness of tissue to hormones that regulate growth and adiposity. The association of ETH10 genotypes with the phenotype of weaning weight provide additional support for utilizing STAT6 as a candidate gene in studies of animal growth.

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Table 1. Genotypic frequency percents of individuals (≥ 99% Red Angus) possessing ETH10 genotype in RAAA database.

Genotype	n	Frequency %
207/217	1	0.02
209/223	2	0.04
209/219	1	0.02
209/223	1	0.02
211/217	2	0.04
215/215	103	2.04
215/217	767	15.16
215/219	113	2.23
215/221	290	5.73
215/223	97	1.92
217/217	1335	26.39
217/219	442	8.74
217/221	920	18.19
217/223	371	7.33
219/219	37	0.73
219/221	169	3.34
219/223	62	1.23
221/221	172	3.40
221/223	139	2.75
221/225	1	0.02
223/223	33	0.65

Table 2. Least squares means for growth and carcass phenotypes in Red Angus cattle (n = 5,058) possessing genotypes containing the 217 allele.

Item	215/217	217/217	217/219	217/221	217/223	SEM	P > F
Birth weight, kg.	35.85	36.10	36.57	36.07	36.34	0.33	0.2283
205-d weight, kg.	264.08[a]	264.95[a]	271.43[b]	266.37[a]	267.90[ab]	2.98	0.0700
365-d weight, kg.	442.56	441.73	445.83	438.62	444.34	4.30	0.4896
LM area, cm^2	77.64	77.35	77.51	75.68	78.24	1.83	0.2494
12^{th} rib fat thickness, cm	0.57	0.57	0.61	0.54	0.58	0.04	0.1436
Intramuscular fat, %	3.69	3.74	3.73	3.71	3.65	0.18	0.9695
Rump fat, cm.	0.71	0.73	0.73	0.73	0.74	0.05	0.8415
LM area/BW, cm^2/kg	0.18	0.18	0.18	0.17	0.18	0.003	0.1383
ADG, kg/d	1.12	1.09	1.08	1.06	1.09	0.02	0.1572

[ab]Within a row, means without a common superscript differ ($P < 0.01$).

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CAMELINA MEAL AND CRUDE GLYCERIN AS FEED SUPPLEMENTS FOR DEVELOPING REPLACEMENT BEEF HEIFERS

P. Moriel, P. L. Price, V. Nayigihugu, and B. W. Hess
Department of Animal Science, University of Wyoming, Laramie 82071

ABSTRACT: Ninety-nine Angus × Gelbvieh rotationally crossed heifers were used in a randomized complete block designed experiment to determine the effect of feeding camelina biodiesel co-products (meal and crude glycerin) on concentration of fatty acids in plasma. Heifers were stratified by BW (300 ± 2.1 kg initial BW) and randomly assigned to receive 1 of 3 experimental supplements (12.6% dietary CP): control (50% ground corn and 50% soybean meal, as-fed); camelina meal (mechanically extracted); and glycerin (50% soybean meal, 33% ground corn, 15% crude glycerin, 2% corn gluten meal; as-fed). Bromegrass hay and supplements were offered daily at 2.40% and 0.3% of BW (as-fed) for the first 30 days and at 2.26% and 0.29% of BW (as-fed) for last 30 days, respectively. Blood samples were taken from the jugular vein at the beginning, middle (30 days) and end (60 days) of the experimental feeding period. Concentration of fatty acids of freeze-dried plasma was determined by GLC. Plasma concentration of identified fatty acids did not differ between heifers fed control and glycerin either at the beginning or end of the experimental feeding period. Except for 18:0 ($P < 0.001$; 3.13, 2.88, and 2.90 ± 0.07 mg of fatty acid/g of freeze dried plasma for camelina meal, control and glycerin, respectively), initial concentrations of plasma fatty acids did not differ (P = 0.135 to 0.973) among dietary treatments. However, the magnitude of difference between heifers fed camelina meal and the other two supplements (5.30 vs. 3.90 ± 0.14 mg of fatty acid/g of freeze-dried plasma) was much greater at the end of the experiment. With the exception of 14:0 (P = 0.108), 14:1 (P = 0.457), and 15:1 (P = 0.471), plasma concentration of total and individual fatty acids was greater ($P \leq 0.039$) in heifers fed camelina meal compared with heifers fed the control or glycerin supplement. Feeding supplemental camelina meal was an effective strategy to increase plasma fatty acid concentrations of developing replacement beef heifers.
Keywords: fatty acids, camelina, glycerin, beef heifers

Introduction

Camelina (*Camelina sativa* L. Crantz) may be an alternative oilseed for biodiesel production due to its simple cultivation, flexible capacity of growing in different climate and soil conditions, no requirements for pesticides application (Zubr, 2003), and the seeds contain 40% oil (Agegenehu and Honermeier, 1997).

Two co-products can be generated from using camelina seeds for biodiesel production. Camelina meal is results from pressing the seeds for oil extraction. The meal contains about 13% fiber, 5% minerals, 45% crude protein (Bonjean and Le Goffic, 1999), and 10% oil with 28.5% 18:2*n*-6 and 41.3% 18:3*n*-3 (Hurtaud and Peyraud, 2007). Crude glycerin is another co-product resulting from biodiesel production. Glycerol, the main compound in crude glycerin, is a liquid substance of sweet taste and high energy concentration (Fisher et al., 1971; 1973; Sauer et al., 1973). Glycerol is extensively fermented in the rumen (Kijora et al., 1998) and increases molar proportions of propionate and butyrate (Khalili et al., 1997). Schröder and Südekum (1999) noted that, regardless of purity, crude glycerin could be fed at up to 10% of dietary DM without compromising digestibility.

It was hypothesized that camelina co-products could be used as substitutes for a conventional corn-soybean meal supplement. Because intestinal disappearance of many 18-carbon fatty acids also increased by feeding camelina (Price et al., 2008), another hypothesis was that feeding camelina co-products would be an effective strategy to increase the animal's fatty acid status. Our objective was to determine the effect of replacing supplemental corn and soybean meal with camelina co-products (meal and crude glycerin) on plasma concentrations of fatty acids of developing replacement beef heifers.

Materials and Methods

General. All procedures for the following experiment were approved by the University of Wyoming Animal Care and Use Committee. Ninety-nine Angus × Gelbvieh rotationally crossed heifers were stratified by BW (300 ± 2.1 kg initial BW). Heifers within each BW block were then allotted randomly to 1 of 15 pens (6 to 7 heifers/pen). Heifer BW was recorded as the average pre-feeding live weights taken on 2 consecutive d at the beginning (February 25 and 26), middle (March 25 and 26), and end (April 25 and 26) of the experimental feeding period.

Diets. Diets were formulated to be isonitrogenous and to provide 12.6% crude protein. Bromegrass hay was offered daily at 2.40% of average BW (as-fed) from February 26 through March 26 and at 2.26% of average BW (as-fed) from March 27 through April 25. Within respective BW blocks, heifers were offered 1 of 3 experimental supplements (Table 1 and 2): a **control** supplement consisting of 50% ground corn and 50% soybean meal (as-fed); mechanically extracted **camelina meal**; and a **glycerin** supplement consisting of 50% soybean meal, 33% ground corn, 15% crude

glycerin, and 2% corn gluten meal (as-fed). Supplements were offered daily at 0.3% of average BW (as-fed) from February 26 through March 26 and at 0.29% of average BW (as-fed) March 27 through April 25. No feed remained in the bunks after each 24-h ration was delivered. Heifers had free access to water and trace mineralized salt [Ultra Balance Spring & Summer Mineral, Hergert Milling Inc., Scottsbluff, NE; guaranteed analysis (percentage of DM): NaCl, 14 to 16; Ca, 18 to 20; P, 8; Mg, 2.5; K, Co, Cu, I, Mn, Zn and Se, less than 1) throughout the experiment.

Table 1. Chemical composition of supplements[1] and the hay fed to developing replacement heifers.

Ingredients	Control	Camelina	Glycerin	Hay
DM, %	91.09	91.44	82.52	92.45
IVDMD, %	92.55	70.57	93.78	61.21
% of DM				
OM	93.11	94.74	94.59	91.64
NDF	12.58	44.48	9.83	60.45
ADF	7.27	27.89	5.93	34.97
CP	43.16	44.74	33.92	9.69
Mcal/kg of DM[2]				
DE	4.07	3.10	4.12	2.69
ME	3.33	2.54	3.38	2.20
NE_m	2.30	1.64	2.34	1.34
NE_g	1.60	1.03	1.63	0.76

[1]Supplements (as-fed) consisted of 50% ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).
[2]Calculated based on equations from NRC (1996) using IVDMD percentage as an estimate of TDN.

Synchronization. On April 26, a 5 mL (25 mg) i.m. dose of prostaglandin (Lutalyse®, Pfizer Animal Health, US) was administered to all heifers. Heifers were combined into 1 large group where they had free access to water, trace mineralized salt (described previously), and bromegrass hay. Estrous activity was evaluated twice daily, and any heifer showing estrus was artificially inseminated 12 h after standing heat. After 10 d from first shot (May 6), a second i.m. injection of prostaglandin (5 mL; 25 mg) was administered to all heifers that did not exhibited estrus. Again, heifers showing estrus were artificially inseminated 12 h after standing heat. Heifers not exhibiting estrus were given an i.m. injection of GnRH (2 mL; 100 µg; i.m; Fertagyl, Intervet, Inc., Millsboro, DE) and bred via AI on May 10. Any heifer showing estrus up through10:00 a.m. on June 1 was again bred via AI 12 h after standing heat. Conception to initial AI was assumed to have occurred if a heifer was not observed in estrus for a second time.

Blood Sampling and Laboratory Analysis. On the second weigh d at the beginning (February 26) and end (April 26) of the experiment, preprandial blood samples were taken from each heifer's jugular vein. Blood samples were collected into 10-mL, EDTA-coated, glass Vacutainer tubes (Becton, Dickson and Co., Franklin Lakes, NJ). Blood samples were placed on ice immediately after collection and then were stored at 4º C for 12 h. Samples were centrifuged at 2500 x *g* for 20 min, the resulting plasma was decanted, and plasma was stored at -20ºC until being analyzed for fatty acids.

Plasma samples were lyophilized (Genesis SQ 25 Super ES Freeze Dryer, The Virtis Co., Gardiner, NY), ground with a mortar and pestle, and fatty acid methyl esters were prepared as described by Lake et al. (2006). Separation of fatty acid methyl esters was achieved by GLC (Model 6890 series II, Hewlett-Packard, Avondale, PA) with a 100-m capillary column (SP-2560, Supelco, Bellefonte, PA), with He as the carrier gas at 0.5 mL/min. The oven temperature was maintained at 175°C for 40 min and ramped to 240°C at 10°C/min. Injector and detector (flame ionization) temperatures were 250°C. Identification of peaks was accomplished with purified standards (Nu-Check Prep, Elysian, MN; Matreya, Pleasant Gap, PA).

Statistical analysis. Data were analyzed as a randomized complete block designed using the PROC GLM procedure of SAS (Version 8.0, 1998, SAS Inst., Inc., Cary, NC) with each pen as the experimental unit.

Table 2. Fatty acid composition of supplements[1] offered to developing replacement heifers.

Fatty acids[2], % of total fat	Control	Camelina	Glycerin
16:0	4.38	8.77	3.62
18:1c9	5.91	16.93	4.57
18:2*n*-6	16.6	24.25	13.3
18:3*n*-3	0.56	35.15	0.51

[1]Supplements (as-fed) consisted of 50% ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).
[2]Estimated based on composition of typical corn (Duckett et al., 2002), camelina meal (Hurtaud and Peyraud, 2007), and soybean meal (Turner et al., 2008).

Results and Discussion

Except for 18:0 ($P < 0.001$), initial concentrations of plasma fatty acids did not differ ($P = 0.135$ to 0.973) among dietary treatments (data not shown). The magnitude of difference between heifers fed camelina meal and the other two supplements was much greater at the end of the experiment. Therefore, initial concentrations of plasma fatty acids were not included as covariates in the analysis of plasma fatty acid concentrations at the conclusion of the experiment.

With the exception of 14:0 ($P = 0.108$), 14:1 ($P = 0.457$), and 15:1 ($P = 0.471$), plasma concentration of total and individual fatty acids at the end of experiment were greater ($P \leq 0.039$) in heifers fed camelina meal compared with heifers fed the control or glycerin supplements (Table 3). This response was expected because Price et al. (2008) showed that lambs fed camelina seeds had a greater percentage of total fatty acids digested in the small intestine compared with lambs fed a diet without supplemental fat. Moreover, concentrations of fatty acids in plasma are directly proportional to the amount of fatty acids

absorbed from the small intestine (Noble et al., 1972).

Table 3. Concentration of fatty acids in plasma of beef heifers following a 60-d feeding period[1].

Fatty acids	Control	Camelina	Glycerin	SEM	*P* value
	mg of fatty acid/g of freeze dried plasma				
14:0	0.10	0.13	0.15	0.010	0.1080
14:1	0.13	0.16	0.15	0.010	0.0030
15:0	0.20[a]	0.26[b]	0.20[a]	0.010	0.0030
15:1	0.21	0.23	0.23	0.001	0.4710
16:0	2.40[a]	3.00[b]	2.40[a]	0.090	0.0030
16:1*t*9	0.31[a]	0.61[b]	0.33[a]	0.020	0.0001
16:1*c*9	0.30[a]	0.35[b]	0.29[a]	0.010	0.0390
17:0	0.24	0.24	0.24	0.010	0.9150
17:1	0.03[a]	0.10[c]	0.05[a]	0.006	0.0001
18:0	3.90[a]	5.30[b]	3.90[a]	0.140	0.0001
18:1*t*11	0.26[a]	0.59[b]	0.25[a]	0.020	0.0001
18:1*c*9	2.10[a]	4.20[b]	2.00[a]	0.110	0.0001
18:2*n*-6	4.60[a]	5.80[b]	4.70[a]	0.130	0.0005
20:1	0.12[a]	0.20[b]	0.13[a]	0.010	0.0090
18:3*n*-3	2.10[a]	3.50[b]	2.20[a]	0.060	0.0001
20:4*n*-6	0.59[a]	0.73[b]	0.62[a]	0.040	0.0500
20:5*n*-3	0.36[a]	0.58[b]	0.40[a]	0.010	0.0001
Total	20.50[a]	31.00[b]	20.90[a]	0.700	0.0001

[1]Bromegrass hay was offered daily at 2.40% of average BW (as-fed) from February 26 through March 26 and at 2.26% of average BW (as-fed) from March 27 through April 25. Supplements (as-fed) were offered at 0.3% of BW and consisted of 50% ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).

Plasma concentration of 18:0 at the end of experiment was greatest (*P*= 0.0001) for heifers fed camelina meal. The greater magnitude of difference between heifers fed camelina meal and the other two supplements at the end of the experiment compared with the beginning of the experiment was likely associated with extensive ruminal biohydrogenation (Scholljegerdes and Kronberg, 2007) and subsequent digestion (Price et al., 2008) of 18C fatty acids originating from the residual oil found in the camelina meal.

Heifers fed camelina meal had greater concentrations of 18:2*n*-6 (*P* = 0.005), 18:3*n*-3 (*P* = 0.0001), 20:4*n*-6 (*P* = 0.05), and 20:5*n*-3 (*P* = 0.0001), compared with heifer fed control and glycerin supplements. These results are consistent with those reported by Scholljegerdes et al. (2008), who found that postpartum beef cows (around 60 d) fed a whole flaxseed supplement (rich in 18:3*n*-3) had elevated (*P* = 0.04) plasma concentrations of 18:2*n*-6, 18:3*n*-3, and 20:4*n*-6 compared with cows fed a whole soybean supplement (rich in 18:2*n*-6). It would be expected that heifers fed camelina meal would have decreased levels of 20:4*n*-6 in circulation because diets high in 18:3*n*-3 decrease production of 20:4*n*-6 (Mattos et al., 2000). However, it is also possible that 18:2*n*-6 served as a precursor for the synthesis of 20:4*n*-6 (Staples et al., 1998), or due to the extensive biohydrogenation of 18:3*n*-3 (83%; Scholljegerdes and Kronberg, 2007), the amount of 18:3*n*-3 in circulation was not sufficient to inhibit production of 20:4*n*-6.

Moriel et al. (2009) reported that dietary treatment did not affect ADG (*P* = 0.978), final BW (*P* = 0.967), heifers detected in estrus before timed AI (*P* = 0.787), first conception rate for timed AI (*P* = 0.541), overall first conception rate (*P* = 0.945) of the heifers used in this experiment. Thus, unlike literature reviewed by Hess et al. (2003, 2008), the increase in plasma fatty acids for heifers fed camelina meal was not sufficient to impact reproductive performance.

In conclusion, feeding peripuberal heifers a camelina meal supplement effectively altered plasma fatty acid profile. Replacing supplemental corn with 15% of crude glycerin, however, did not affect fatty acids profile of developing replacement beef heifers.

Implications

Camelina co-products (meal and crude glycerin) are suitable replacements for conventional corn-soybean meal supplements when offered to replacement beef heifers for 60 days before breeding.

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INFLUENCE OF STOCKING DENSITY ON GRAZING BEEF CATTLE PERFORMANCE, DIET COMPOSITION, FORAGING EFFICIENCY, AND DIET QUALITY ON A LATE-SPRING EARLY-SUMMER NATIVE BUNCHGRASS PRAIRIE.

S. Wyffels*[1], T. DelCurto[1], A. Clark[1], P. Kennedy[1], R. Taylor[2], S. Debano[3]

[1]Eastern Oregon Agricultural Research Center, Oregon State University, Union, OR, USA, [2]The Nature Conservancy, Enterprise, OR, USA, [3]Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, OR, USA

ABSTRACT: This study evaluated the influence of cattle stocking density on botanical composition of diet, diet preference, and cattle performance on a native bunchgrass prairie. In each of two years, 192 cow-calf pairs (549.27 kg, BCS = 4.89) and 48 yearling heifers (383.34 kg, BCS = 5.02) were stratified by age and body condition, and randomly allotted to a randomized block design (four blocks) with the following treatments: 1) Control, no livestock grazing; 2) low stocking, 0.36 animal units (AU)/ha; 3) moderate stocking, 0.72 AU/ha; and 4) high stocking, 1.08 AU/ha for a 42 d grazing period (late May to early July). Using ruminally-cannulated cows, diet composition and masticate samples were taken, in May and July, following 20 min grazing bouts and were analyzed for forage fiber and crude protein. Treatments had no influence on cattle weight change and body condition ($P > 0.10$). In regard to foraging efficiency, grams per minute and bites per minute were lower in the early collection than the late collection (g/min 21.51 vs. 31.21, bite/min 16.71 vs. 26.71) and higher in the control pasture than the grazed pastures (g/min 31.21 vs. 12.44, bite/min 26.71 vs. 12.70; $P < 0.05$). In addition, grams per bite decreased linearly with increased stocking density ($P < 0.05$). Forage fiber (ADF and NDF) was lower in May compared to July whereas CP was higher in May compared to July ($P < 0.01$). Stocking density did not influence diet quality ($P > 0.20$). Botanical compositions of diets were 91.98% grass and 7.95% forb species, with beef cattle showing strong relative preference for grass (RPI = 1.69) and weak relative preference for forbs (RPI = 0.18). Shifts in diet composition and preference were noted in regard to stocking density with Idaho Fescue, Prairie Junegrass, Hawkweed species and Hoary Balsamroot ($P < 0.05$). In summary, cattle stocking density had minimal effect on diet quality and cattle performance, but did influence foraging efficiency, botanical composition of the diet, and relative preferences of beef cattle grazing on a bunchgrass prairie.

Key Words: Beef cattle, Stocking density, Grazing behavior

Introduction

With increasing global concern about ecological sustainability, it is imperative to understand the impacts of cattle grazing on ecosystem health of western rangelands. According to Belsky and Blumenthal (1997), domestic livestock may alter ecosystem processes by reducing the cover of herbaceous plants and litter, disturbing and compacting soils, reducing water infiltration rates, and increasing soil erosion. These changes in ecosystem processes, in turn, may be related to a loss in vegetation and wildlife diversity. Most grazing studies have focused on plant responses to grazing and those studies that have investigated animal responses suffer from numerous experimental design problems. These include: 1) too small a spatial scale for relevance to wildlife populations; 2) treating cattle as present or absent and ignoring potential stocking density influences; 3) and poor documentation of grazing history. In addition, numerous mechanisms by which herbivores may affect biodiversity have been proposed, but limited research has been conducted.

Therefore, we developed a large-scale multi-disciplinary study using a "food web" approach, to investigate the effects of 4 levels of domestic ungulate herbivory on three trophic levels (plants, aboveground invertebrates, and passerines) in a native bunchgrass community. To this end, our objectives were:

1) Evaluate population- and community-level responses to variation in livestock stocking density;
2) Determine sustainability (both ecological and economic) of varying beef cattle stocking densities.

This paper represents a component of the above large-scale study and is designed to test the effects of cattle stocking density on diet quality, foraging efficiency, botanical composition of diet, and performance. We hypothesize that as stocking density increases, diet quality, performance, and foraging efficiency will decrease, whereas, botanical composition of diets will change in response to species availability.

Materials and Methods

The study was conducted from late May to early July of 2007 and 2008 in northeast Oregon at The Nature Conservancy's Zumwalt Prairie Preserve. The Zumwalt Prairie is located on a basalt plateau at an elevation of 1340 to 1460 m with a mean slope of 7%. This area receives around 330 mm of precipitation annually falling in spring as localized thunderstorms and in winter as snow with a distinct dry period lasting from July through August. The average annual temperature is 6.4°C, and ranges from -

2.8°C in December to 17.1°C in July (Damiran et at. 2007). Vegetation is dominated by native bunchgrass species that include Idaho fescue (*Festuca idahoensis*), prairie Junegrass (*Koeleria macrantha*), and bluebunch wheatgrass (*Pseudoroegneria spicata*) with the main forbs species consisting of old man's whiskers (*Geum triflorum*), twin arnica (*Arnica sororia*) and lupin (*Lupinus spp.*). Shrub species make up a very small portion of the study area and consist of primarily snowberry (*Symphoricarpos albus*) and wild rose (*Rosa spp.*). Total mean annual production for vegetation across the study area ranges from 1262 to 1928 kg/ha (Darambazar et al. 2007).

This study was conducted as a randomized complete block design with four grazing treatments. The total study area consisted of 640 ha that was fenced into four 160 ha blocks each containing four 40 ha pastures. The four grazing treatments (control, low, moderate, and high) were randomly assigned to each pasture within each block. Within each pasture, 36 monitoring sites were established uniformly along a grid of 6 north to south and 6 east to west transects using global positioning systems (GPS).

In each of the two years, 192 cow-calf pairs (549.27 kg, BCS = 4.89) and 48 yearling heifers (383.34 kg, BCS = 5.02) were stratified by age and body condition, and randomly allotted to the following treatments: 1) control, no livestock grazing; 2) low stocking, 0.36 animal units (AU)/ha; 3) moderate stocking, 0.72 AU/ha; and 4) high stocking, 1.08 AU/ha for a 42 day grazing period. Treatments were derived by setting the historic stocking densities for the Zumwalt Prairie as moderate, low was 50% of the moderate stocking density and high was 150% of the moderate stocking density.

Measurements taken pre- and post grazing included cow weights, body condition and calf weights. Post treatment utilization estimates were obtained by means of ocular estimates similar to that described by Parsons et al. (2003) using regression to adjust for observer error.

Data was collected on standing crop by species in late spring to early summer of 2006, prior to initiation of grazing treatments. This information was used to determine the relative preference of species consumed by cattle in each treatment. Cattle dietary composition information was collected in late May and early July from six cows in each experimental unit using bite-count methodology similar to that described by Wickstrom et al. (1984) and Canon et al. (1987). Diet composition data were collected in control pastures prior to the initiation of the grazing treatments and in each unit after the grazing treatment was applied. Data was collected by observing six ruminally-cannulated cows for 20 min grazing bouts in each pasture. Each observer was assigned a cow at random for each grazing bout. The animals were accustomed to observers in close proximity and observers had prior training in plant identification. Observers used a small hand-held tape recorder to record the number of bites of each plant species consumed (Findholt et al. 2004). The data was then transcribed from the tapes and placed in an excel file for analysis. Relative preference was calculated by % diet composition divided by % forage composition.

Cattle diet quality information was collected concurrently with diet composition measurements. Each cow's ruminal contents were evacuated and the ruminal wall washed with a sponge to remove remaining digesta and ruminal fluid. Cows were allowed to graze for 20 minutes and diet samples were obtained via the ruminal cannula. One rumen evacuation per cow was performed in each experimental unit. Ruminal samples were dried in a forced-air oven (55°C; 96 h) and ground to pass a 1-mm screen in a Wiley mill. Rumen samples were analyzed in duplicate for organic matter, nitrogen (Leco CN-2000; Leco Corporation, St. Joseph, MI), and NDF and ADF (Ankom 200 Fiber Analyzer, Ankom Co., Fairport, NY).

Foraging efficiency was calculated by dividing the dried masticate sample weight by 20 to determine grams consumed per minute. Bites consumed per minute was calculated by dividing the total number of bites per grazing bout by 20 and grams consumed per bite was calculated by dividing total grams of the sample by the total amount of bites per 20 min grazing bout.

Data was analyzed using GLM procedures of SAS (2002). Predetermined contrast statements were used to determine treatment differences (Steel et. al 1997). Contrast statements were: 1) May control vs. July control, 2) July control vs. grazed, 3) linear grazing effect, and 4) quadratic grazing effect. Differences were considered significant at $P < 0.05$.

Results and Discussion

Cattle across all treatments showed no difference in weight change or body condition (Table 1; $P > 0.10$). Calf average daily gains also showed no differences between treatments ($P > 0.80$) averaging 1.40 kg /d. Cattle stocking appeared to have no influence on performance during the 42 d grazing periods.

Grass made up the greatest proportion of all cattle diets (Table 2; 91.98%), which agrees with other regional studies by Miller and Krueger (1976) and Walburger et al. (2007). Percent grasses and forbs found in the diet were similar across all treatments ($P > 0.35$). Grass was preferred to forbs across all treatments as determined using a relative preference index (Table 3). Cattle displayed a strong preference for grass regardless of treatment and stocking rate (RPI = 1.69). Forbs were never preferred as a forage class in any treatment (RPI = 0.18). Although forbs as a forage class were never preferred some individual forbs species were preferred, including Canadian Milkvetch (*Astragalus canadensis*), Hoary Balsamroot (*Balsamorhiza incana*) and Hawkweed spp (*hieracium spp*).

Prairie junegrass, hoary balsamroot and onespike oatgrass (*Danthonia unispicata*) were consumed in larger percentages in May than in July ($P < 0.04$). Sandbergs bluegrass (*Poa secunda*), brome spp (*Bromus spp*; combination of smooth brome and California brome) and hoary balsamroot all tended to be preferred in May compared to July diets ($P < 0.10$). California oatgrass (*Danthonia Californica*), Idaho fescue and bluebunch wheatgrass were all consumed in larger amounts in July than they were in May ($P < 0.04$). California oatgrass and timothy (*Phleum pratense*) tended to be preferred in July

rather than May ($P < 0.10$). Differences in cattle preferences and diet consumption from May to July may be attributed to the physiological stages of each species. Forage nutritive quality and palatability is reduced substantially as plants become dormant or in advanced phenologic stages (Holechek et al 2001). According to Vallentine (1990) palatability is a key factor in what stimulates animals to prefer one forage over another. Cattle may have shifted in diet composition and preference due to some species entering dormancy while others were still actively growing.

Hawkweed spp. and prairie junegrass were consumed in higher amounts in control pastures than in grazed pastures ($P < 0.04$). Hawkweed spp., timothy and annual hairgrass (*Deschampsia danthonioides*) tended to be preferred in the control pastures than in grazed pastures ($P < 0.09$). California oatgrass and intermediate wheatgrass (*Thinopyrum intermedium*) tended to be consumed in higher amounts in control pastures than in grazed pastures ($P < 0.07$). Rush spp. (*Juncus spp.*) had a linear decrease in consumption as stocking densities increased ($P < 0.03$). Prairie junegrass tended to have a linear decrease in consumption as stocking rates increased ($P < 0.07$). Selectivity by an animal may be influenced by the presence of other animals in an area by changing the short-term relative availability of the different plant species (Vallentine 1990). Species that were preferred and consumed in high amounts in the control pastures may have already been consumed in grazed pastures resulting in an absence of preferred species as stocking densities increase.

Idaho fescue consumption was higher in the grazed pastures ($P < 0.01$) than in the control, with a linear tendency of increased consumption as stocking densities increased ($P < 0.09$). Idaho fescue was also preferred in grazed pastures than in control pastures ($P < 0.02$). Vallentine (1990) states that as the availability of preferred plants decreases the search time required for consumption may not be effective and alternative species are utilized instead. This may suggest that as preferred species become more difficult to find, cattle may begin to consume higher amounts of abundant non-preferred species at higher stocking densities. In this study, some of the most preferred species in the non-grazed and light stocking density pastures were not dominant grasses in the pastures and represented a minor part of the total vegetation biomass. In addition, several of the "non-native" species (such as Kentucky bluegrass (*Poa pratensis*), intermediate wheatgrass, and timothy) were highly preferred relative to the native vegetation. This observation perhaps raises more questions relative to the long-term sustainability of livestock herbivory in native bunchgrass rangelands and the role of non-native vegetation in those plant communities.

Cattle diets were higher in CP during the May collection as compared to July (Table 4; $P < 0.01$), while NDF and ADF values were lower in May compared to July collections ($P < 0.01$). We found no differences in CP, ADF and NDF between stocking densities ($P > 0.20$). Overall grasses and forbs in the May control pastures had greater nutritive values than grasses and forbs in the July control pastures. This may be because of the higher nutritional value of plants in their younger stages of phenology (Holecheck 2001).

In regard to foraging efficiency, grams per minute and bites per minute were lower ($P < 0.05$) in the early collection than the late collection (Table 4; g/min 21.51 vs. 31.21, bite/min 16.71 vs. 26.71, respectively) and higher in the control pasture than the grazed pastures (g/min 31.21 vs. 12.44, bite/min 26.71 vs. 12.70; $P < 0.05$). In addition, grams per bite decreased linearly with increased stocking density ($P < 0.05$). The overall decrease in foraging efficiency may be due to the inability to find preferred species, which may result in increasing search time and smaller amounts consumed per bite of the preferred species.

Implications

Although no differences in diet quality or cattle performance were found after a 42-day grazing period, foraging efficiency and dietary shifts in botanical composition of diets were observed. This may suggest that if cattle were to remain grazing, differences in diet quality and performance would be expected. Decreased foraging efficiency could ultimately result in cattle spending more time searching for preferred forages resulting in increased grazing time for the same nutrient quality or decreased nutrient quantity with increased stocking density. Eventually this would be reflected on cattle body condition and weight gains.

Cattle also seem to shift the composition of their diets based on time of year and stocking density. These shifts are important to recognize for management of an entire ecosystem. In high stocking densities cattle may overgraze preferred species, which could potentially cause an ecological shift in vegetative communities.

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Table 1. The effects of stocking density on body condition and weight change of cattle grazing on the Zumwalt Prairie, northeast Oregon (Data averaged over 2007 and 2008)

		Stocking density				Preplanned contrasts	
		Low	Mod	High	SE[1]	Linear	Quadratic
Initial	Cow weight, kg	551.95	548.46	547.46	9.98	0.50	0.83
	Cow BCS	4.85	4.92	4.89	0.09	0.77	0.68
	Heifer weight, kg	378.78	382.34	388.92	11.50	0.20	0.82
	Heifer BCS	4.97	5.02	5.09	0.044	0.09	0.82
	Calf weight, kg	134.26	134.54	134.56	6.49	0.93	0.96
Change	Cow weight, kg	24.24	23.41	24.70	7.43	0.93	0.80
	Cow BCS	0.48	0.45	0.39	0.08	0.48	0.85
	Heifer weight, kg	50.05	42.92	39.82	10.40	0.16	0.74
	Heifer BCS	0.56	0.53	0.41	0.06	0.11	0.55
	Calf gain ADG kg/d	1.41	1.39	1.39	0.09	0.81	0.88

[1]Standard Error (Pooled) N=4

Table 2. Percent diet composition by forage class and the top 95% of species consumed by cattle grazing at stocking densities of 0%, low, moderate, and high at the Zumwalt Prairie, northeast Oregon (Data averaged over 2007 and 2008).

	Control		Stocking density				Preplanned contrasts			
							Control	Stocking density		
	May	July	Low	Mod	High	SE[1]	May vs. July	Control vs. grazing	Linear	Quad[2]
% Grass	**90.54**	**94.36**	**93.78**	**91.28**	**89.95**	**2.86**	**0.37**	**0.43**	**0.36**	**0.87**
Idaho Fescue	2.23	9.42	28.18	40.72	40.27	4.62	0.03	0.01	0.09	0.27
Kentucky Bluegrass	26.13	19.50	21.98	15.95	15.86	4.83	0.36	0.78	0.38	0.62
Bluebunch Wheatgrass	7.45	21.08	24.74	14.68	15.40	4.12	0.04	0.56	0.13	0.30
Brome spp*	29.80	12.25	5.36	9.82	2.68	7.24	0.12	0.46	0.80	0.52
Inter. Wheatgrass	0.19	9.22	0.91	0.44	0.00	3.59	0.12	0.05	0.86	0.99
Onespike Oatgrass	10.82	3.57	0.42	0.56	0.77	2.20	0.04	0.26	0.91	0.99
Timothy	0.18	1.94	1.36	2.66	3.72	1.43	0.41	0.70	0.26	0.95
Prairie Junegrass	0.83	2.32	1.68	0.51	0.39	0.46	0.04	0.02	0.07	0.36
Sandberg Bluegrass	3.12	1.02	1.49	0.78	2.96	0.96	0.15	0.52	0.29	0.24
California Oatgrass	0.00	2.49	0.97	0.76	0.74	0.68	0.03	0.05	0.82	0.91
Rush spp	2.075	0.07	3.23	0.53	0.00	0.96	0.17	0.30	0.03	0.37
%forb	**9.44**	**5.60**	**6.11**	**8.66**	**10.04**	**2.88**	**0.37**	**0.43**	**0.35**	**0.87**
Hoary Balsamroot	6.63	2.90	1.69	0.27	0.46	1.14	0.04	0.14	0.46	0.57
Hawkweed spp	0.84	1.62	0.37	0.15	0.42	0.45	0.25	0.03	0.93	0.67

*Combination of California Brome and Smooth Brome

[1]Standard Error (Pooled) N=4; [2]Quadratic contrast

Table 3. Relative preference index by forage class, dominant grasses and forbs consumed by cattle grazing at 0%, low, moderate, and high stocking densities at the Zumwalt Prairie, northeast Oregon (Data averaged over 2007 and 2008).

	Control		Stocking density				Preplanned contrasts			
							Control	Stocking density		
	May	July	Low	Mod	High	SE[1]	May vs. July	Control vs. grazing	Linear	Quad[2]
RPI Grass	**1.73**	**1.80**	**1.57**	**1.57**	**1.77**	**0.13**	**0.70**	**0.30**	**0.30**	**0.56**
Idaho Fescue	0.11	0.44	1.56	3.85	2.55	0.68	0.74	0.02	0.32	0.05
Bluebunch Wheatgrass	0.70	1.63	1.41	1.45	1.45	0.40	0.14	0.68	0.93	0.96
Kentucky Bluegrass	5.84	4.18	3.55	1.74	2.24	1.16	0.34	0.23	0.43	0.43
Prairie Junegrass	0.18	0.52	0.39	0.10	0.09	0.09	0.02	0.01	0.03	0.25
Brome spp*	15.77	7.02	11.77	2.79	4.08	3.40	0.10	0.84	0.13	0.24
Annual Hairgrass	0.00	5.62	0.09	0.04	0.27	2.51	0.14	0.08	0.96	0.96
Timothy	1.22	8.03	1.18	0.78	3.63	2.58	0.09	0.06	0.51	0.61
Sandberg Bluegrass	8.27	1.62	1.51	1.18	6.04	2.53	0.09	0.67	0.23	0.42
Onespike Oatgrass	205.23	146.87	2.23	5.27	2.62	91.49	0.66	0.20	0.99	0.98
Rush spp	19.68	0.18	6.65	0.46	0.00	8.59	0.14	0.83	0.59	0.79
Inter. Wheatgrass	0.26	32.90	35.50	0.57	0.00	16.70	0.20	0.30	0.16	0.41
California Oatgrass	0.00	12.02	7.43	0.94	5.69	4.50	0.09	0.18	0.79	0.32
RPI Forb	**0.21**	**0.13**	**0.16**	**0.21**	**0.19**	**0.07**	**0.44**	**0.51**	**0.76**	**0.69**
Canadian Milkvetch	0.10	0.07	1.14	0.48	1.19	0.65	0.98	0.27	0.96	0.40
Twin Arnica	0.01	0.01	0.00	0.01	0.00	0.01	0.18	0.70	1.00	0.22
Western Yarrow	0.05	0.05	0.24	0.09	0.11	0.05	0.93	0.09	0.08	0.21
Hawkweed spp	1.09	3.01	0.37	0.13	1.46	1.05	0.23	0.07	0.48	0.55
Old Man's Whiskers	0.02	0.01	1.16	0.21	0.40	0.41	0.99	0.24	0.21	0.27
Lupin spp	0.03	0.01	0.01	0.03	0.05	0.02	0.36	0.30	0.12	0.77
Cinquefoil spp	0.02	0.05	0.10	0.79	0.43	0.22	0.93	0.15	0.32	0.08
Hoary Balsamroot	4.58	1.23	0.56	0.15	0.20	1.26	0.09	0.53	0.84	0.88

*Combination of California Brome and Smooth Brome
[1]Standard Error (Pooled) N=4; [2]Quadratic contrast

Table 4. Percent crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of masticate samples and bites per min, grams per bite and grams per min from cattle grazing 0%, low, moderate, and high stocking densities at the Zumwalt Prairie, northeast Oregon (Data averaged over 2007 and 2008).

	Control		Stocking density				Preplanned contrasts			
							Control	Stocking density		
	May	July	Low	Mod	High	SE[1]	May vs. July	Control vs. grazing	Linear	Quadratic
Diet Quality										
CP	15.82	10.46	10.08	10.59	10.51	0.34	<0.01	0.87	0.39	0.49
NDF	53.92	62.53	62.08	62.24	61.95	0.73	<0.01	0.61	0.90	0.80
ADF	33.34	40.30	40.82	41.15	40.03	0.57	<0.01	0.58	0.34	0.32
Foraging Efficiency										
Bites/min	16.71	26.71	13.56	11.81	12.73	2.52	0.02	<0.01	0.82	0.67
G/bite	1.58	1.18	1.17	1.08	1.10	0.19	0.18	0.60	0.04	0.19
G/min	21.51	31.21	14.61	11.11	11.60	2.9	0.04	<0.01	0.48	0.58

[1]Standard Error (Pooled) N=4

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ANALYSIS OF ESTRONE SULPHATE, TESTOSTERONE, AND CORTISOL CONCENTRATIONS AROUNDTIME OF EJACULATION ANDPOTENTIAL CORRELATION TO SEXUAL BEHAVIOR ANDSPERM CHARACTERISTICS IN STALLIONS

J. L. Seale, G. Briers, D. H. Sigler, C. A. Cavinder
Texas A&M University, College Station, TX

ABSTRACT: In the stallion, inconsistent sexual behavior and variable semen quality are common, and this variability has been attributed to differences in circulating hormone concentrations. The objectives of this study were to quantify the circulating concentrations of **testosterone (T), cortisol (C)** and **estrone sulphate (ES)** around ejaculation, and determine potential effects of blood hormone concentrations on sexual behavior and sperm characteristics. Miniature stallions (n=7) were observed for sexual behavior and semen characteristics. Blood was drawn at sequential times around ejaculation, including 15 min before, immediately following and 15, 30, and 60 min after ejaculation. Plasma was later analyzed for concentrations of T, ES and C. Semen was evaluated for volume, sperm concentration and progressive motility. Sexual behavior was quantified by assigning a libido score to each stallion, recording reaction time and the number of jumps required for ejaculation. Data were analyzed using two-way ANOVA and regression analysis. Both ES and C increased at the time of semen collection, while T did not ($P < 0.05$). Estrone sulphate and the ratio of ES to T were negatively correlated to libido scores ($P < 0.05$). Additionally, a positive relationship was found between ES and reaction time, as well as between C and libido scores ($P < 0.05$). No relationship was observed between T and sexual behavior; however T was positively correlated and the ratio of ES to T was negatively correlated to progressive motility ($P < 0.05$). No other association was detected between ejaculate parameters and hormone concentrations. These results enhance our understanding of stallion hormone profiles, and also provide further insight into the hormonal control of stallion sexual behavior and sperm production.

(**Key Words**: Stallion, Sexual Behavior, Testosterone, Estrone Sulphate, Cortisol)

INTRODUCTION

The neuroendocrine system is responsible for control of the reproductive organs through the secretion of hormones. Consequently, in many species reproductive variability among males has been attributed to differences in circulating hormone concentrations. However, the function and relationships between reproductive hormones in the stallion is not completely understood.

Circulating T concentrations increase during the natural breeding season, and at the same time the stallion becomes more sexually active (Thompson *et al.*, 1977). However, differences in circulating blood concentration of T could not be linked to differences in libido in stallions (Burns et al., 1985) or bulls (Price *et al.*, 1986).

The sulfated form of estrone is the most abundant plasma estrogen in the stallion and its concentration is almost 100 times that of T (Bono *et al.*, 1982). Thus, it can be speculated that, due to uniquely high concentration, ES may play a central role in stallion reproductive behavior and physiology.

Cortisol may affect reproductive endocrinology through its interaction with T. Increasing blood C concentration in the stallion has produced varying results, causing both an increase (Villani *et al.*, 2006) and a decrease in T concentration (Wiest *et al.*, 1988; Rabb *et al.*, 1989). Still, in the bull C and T do not interact (Henney *et al.*, 1990). Inconsistent results leave the association between C and T unclear.

Due to variability in previous results, the objectives of this study were to quantify the circulating concentrations of T, ES and C around ejaculation and to determine potential effects of blood hormone concentrations on sexual behavior and sperm characteristics.

MATERIALS AND METHODS

Miniature stallions (n=7) between the ages of 3 and 13 y were observed. They were housed in individual stalls at the Texas A&M Horse Center and had no visual contact with mares. Each stallion was fed 1.5% of BW in alfalfa hay/d and 0.5% of BW in concentrate/d with *ad libitum* access to fresh water. Horses used in this study were maintained under the approval of the Texas A&M University Institutional Agricultural Animal Care and Use Committee using guidelines set forth by the Federation of Animal Science Societies (1999).

Semen was collected from each stallion once a day for 3 consecutive days in order to provide a more uniform ejaculate on the third collection, followed by 11 days of sexual rest. The first 2 collections were discarded and ejaculate gel-free volume, concentration, and motility of the third collection were recorded. A total of 5 ejaculates, collected over 55 days from August to October, were evaluated from each stallion. Collection was achieved using an artificial vagina (AV, Missouri model). Upon collection the gel portion of the ejaculate was filtered off in order to measure the volume of the sperm rich gel-free portion. Spermatozoa concentration in the ejaculate was measured using a densimeter (Animal Reproduction Systems, Chino, CA). The sample was then extended using a commercial extender (INRA-96® Breeder's Choice,

Aubrey, TX) to achieve a concentration of 25 to 50 x 10^6 spermatozoa/mL, which is consistent with accurate analysis of progressive motility using computer-assisted semen analysis (CASA, Ceros Motility Analyzer, Hamilton-Thorne, Beverly, MA). The samples were maintained at 37°C until analysis.

Libido scores (Table 1) were assigned by 2 individual appraisers to each stallion when presented with an estrus mare in order to quantify the intensity of sexual arousal.

Table 1. Libido score description

Score	Description
0	no interest in an estrus mare
1	slight vocalization and interest initially but quickly fades
2	moderate vocalization and interest in mare however interest dissipates
3	moderately interested with consistent contact with mare
4	highly interested with vocalization and squealing, consistent contact or attempt to mount

Additionally, the number of mounts required before ejaculation and the reaction time for each stallion was recorded. Timing began when the stallion entered the breeding facility and a time was recorded upon ejaculation.

In order to measure the changes in circulating hormone concentrations around copulation, blood was collected via jugular venipuncture using vacutainers. Blood sampling took place on each third consecutive semen collection 15 min prior to entering the breeding facility, immediately following ejaculation, and 15, 30 and 60 min following ejaculation. Blood was centrifuged at 2500 rpm for 20 min, in a refrigerated centrifuge. Blood plasma was then collected and stored in micro centrifuge tubes at -20°C until assayed for T, C and ES by radioimmunoassay.

Data were subjected to one-way ANOVA to determine differences between stallions in all parameters measured (T, ES, C, ES:T, concentration, volume, progressive motility, number of jumps, reaction time and libido score). A two-way ANOVA was used to determine the differences in hormone concentrations over time. Correlation coefficients were calculated using regression analysis to determine relationships between sexual behavior, semen parameters and hormone concentrations. Hormone concentrations in the stallion vary throughout the year and because data were collected over a period of 3 months, ANOVA was used to determine if date had an effect on hormone concentrations. All statistical analyses were performed with SPSS computer software (SPSS Inc., 16.0).

RESULTS

Mean blood T concentration was not significantly different at any measured point from pre-mating to 60 min after ejaculation (Figure 1). Mean ES concentration showed a significant increase immediately following ejaculation (from 116.18 ± 51.16 ng/ml to 209.88 ± 82.49 ng/ml; $P < 0.001$). Concentrations then returned to basal values within 30 min following ejaculation (Figure 2). Mean C concentrations rose significantly from pre-mating values until 15 min following ejaculation (from 4.31 ± 1.31 μg/dL to 4.92 ± 1.63 μg/dL; $P < 0.05$). Concentrations then returned to pre-mating values within 30 min following ejaculation (Figure 3).

Estrone sulphate and the ratio of ES to T were negatively correlated ($P < 0.05$) to stallion libido score at times -15, +30 and +60, when ES concentration was again approaching pre-mating values (Table 1). Testosterone, however, was not related ($P > 0.05$) to libido score at any measurement time (Table 1). Cortisol concentration was positively correlated to libido score at times -15, 0, and +15 (Table 1). Estrone Sulphate concentration was also positively correlated ($P > 0.05$) to reaction time at times -15 and +60 (Table 3). No significant correlations were found between number of jumps and hormone concentrations at any time measured.

Testosterone at time -15 was positively correlated to progressive motility, and ES/T was negatively correlated to progressive motility (Table 3). No significant relationships were observed between hormone concentrations and sperm concentration or volume of the ejaculate (Table 3).

DISCUSSION

Testosterone was measured both before and after ejaculation to determine its importance in the change of stallion behavior when presented with an estrus mare. No significant differences were observed between time periods (-15, 0, +15, +30 and +60). This is in agreement with previous research which found no change of T when stallions were presented with an estrus mare or upon ejaculation (Cox and Williams, 1975). The lack of significant variation in T at any time surrounding mating leads to the possibility that T may not play a major role in stallion sexual behavior. Additionally, there were no significant ($P > 0.05$) relationships between T and libido scores, reaction times, or number of jumps. This theory is in agreement with several other studies which stated that T concentration could not be linked to libido (Burns *et al.*, 1985; Price *et al.*, 1986). However, previous reports state that sexual performance could be restored when castrated males were administered exogenous T (Thompson *et al.*, 1980). This anomaly may be due to the fact that T can be

converted to estrogens. When geldings were administered T alone, an increase in libido was observed, and upon subsequent blood collections both T and estrogens were observed (Thompson *et al.*, 1980); thus demonstrating that T can be aromatized to estrogen in the horse. Further, when castrated male rats were administered T, an increase in the desire to mount was observed; however, when T was given in conjunction with an aromatization inhibiting steroid, the increase in libido was not observed (Christensen and Clemens, 1975). These results indicate that the aromatization of T to estrogens, and not T alone, may be responsible for the changes in stallion sexual behavior.

Ejaculate parameters were also compared to T to ascertain if any association exists. Testosterone was not related to volume or sperm concentration. However, a positive correlation was observed between T and progressive motility. This same relationship was observed when testicular T was suppressed in the stallion (Squires *et al.*, 1981).

When measured at times surrounding mating, mean ES concentration showed a significant increase immediately following ejaculation, time 0 (from 116.18 ± 51.16 ng/ml to 209.88 ± 82.49 ng/ml; $P < 0.001$). Concentrations then returned to basal values within 30 min following ejaculation. These data are consistent with prior results for stallions (Bono *et al.*, 1982; Villani *et al.* 2006). The suppression of ES concentration at times before and after ejaculation leads to the speculation that ES may play a substantial role in stallion sexual behavior. Upon further statistical analysis, ES was found to have a negative correlation with libido scores at times -15, +30, and +60 ($P < 0.05$). Additionally, ES was significantly positively correlated to reaction time 15 min prior to ejaculation and at time +60 ($P < 0.05$). These relationships identify ES as a major factor in stallion sexual behavior.

Neither volume, progressive motility, nor spermatozoa concentration were correlated to ES in the present data. Earlier studies suggested that estrogen concentration was positively correlated to sperm concentration and volume of the ejaculate in the stallion (Roser and Hughes, 1992). Another study reports that estrogens suppress spermatozoa concentration (Thompson and Honey, 1984). However, these studies measured estrone and estradiol-17β in their free form; whereas, in the present study the sulfated form of estrone was measured. The current results indicate that ES has neither a stimulatory nor inhibitory effect on ejaculate volume or concentration.

Previous research indicates that both T and estrogens are required for normal copulation in males of several species (Thompson *et al.*, 1980; Arteaga-Silva *et al.*, 2005). Administration of estrogens to castrated males enhanced sexual arousal; however, intromission and ejaculation were not achieved unless T was also administered. Furthermore, in the bull, low libido was correlated to significantly higher estrogen to T ratios (Henney *et al*., 1990). This same relationship was found in the current data. The ratio of ES/T at times -15, +30, and +60 was negatively correlated to libido scores ($P < 0.05$). The ratio of ES to T was also negatively correlated to progressive motility ($P < 0.05$). However, ES/T was not linked to reaction time or number of jumps. Still, the relationships discovered indicate that pre-mating blood concentrations of ES values and the ratio of ES to T are more accurate predictors of stallion libido and potential motility than T concentrations alone.

Mean C concentrations rose significantly from pre-mating values until 15 min following ejaculation (from 4.31± 1.31 µg/dL to 4.92 ± 1.63 µg/dL; $P < 0.05$). Concentrations then returned to pre-mating values within 30 min following ejaculation. A similar pattern was observed in another study which reported an increase in C concentration from pre-mating values to a peak concentration 10 min following ejaculation (Villani *et al.*, 2006). However, others reported that C remained at peak levels until 120 min post-ejaculation (Tamanini et al., 1983). These sustained high levels were not found in the current study as C returned to basal values within 30 min of mating.

The change in C in the time surrounding ejaculation leads to the theory that C may play a role in stallion sexual behavior. This theory is supported in the present study as libido scores were found to be positively correlated to C at times -15, 0, and +15 ($P < 0.05$). These findings contradict previous research in human and rat males which stated that a negative relationship exists between C and sexual arousal (Sirinathsinghji *et al.*, 1983; Uckert *et al*., 2003). However, in the stallion it has been consistently shown that C increases when sexual arousal is stimulated either by presenting an estrus mare or upon hearing another male being stimulated (Villani *et al*., 2006; Rabb *et al*., 1989; Colborn *et al.*, 1991). These findings in conjunction with the present results lead to the conclusion that, unlike humans and rats, the stallion exhibits a positive relationship between C and libido.

The results of this study will provide further understanding of stallion reproductive physiology and behavior. This additional knowledge could eventually lead to the development of more efficient stallion management practices and ultimately decrease the financial burden of reproductively inconsistent stallions.

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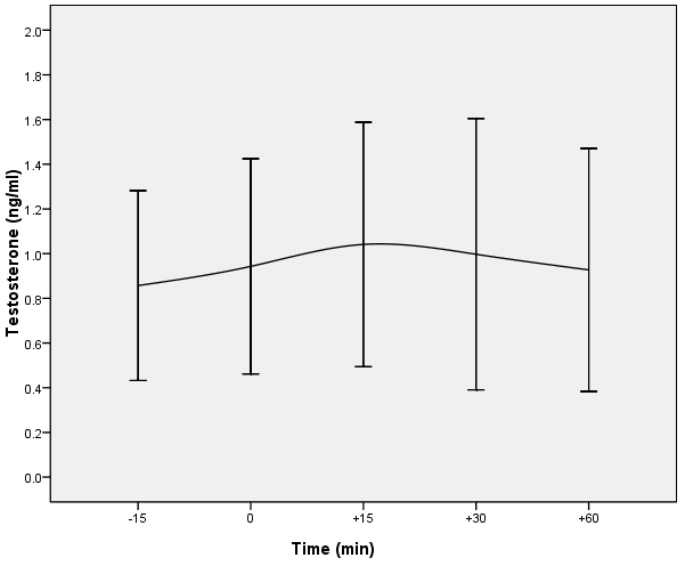

Figure 1. Mean (± SD) testosterone concentration for all stallions for pre-mating (time -15), immediately following ejaculation (time 0), and post-ejaculation periods (times +15, +30, +60).

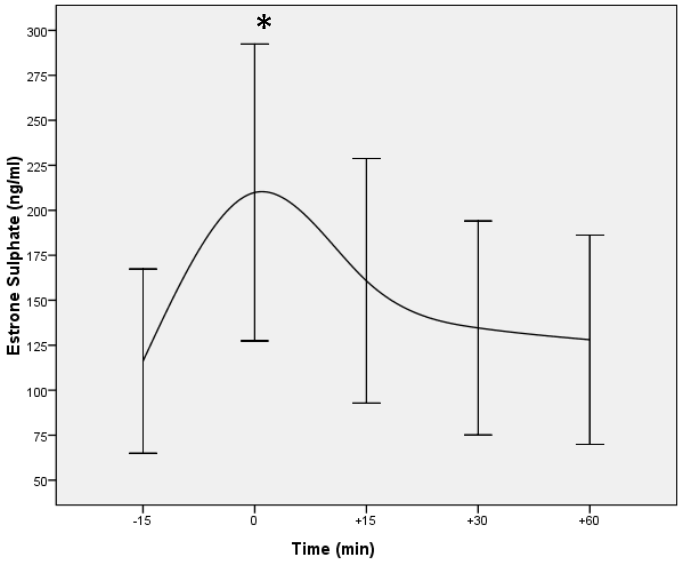

Figure 2. Mean (± SD) estrone sulphate concentration for all stallions for pre-mating (time -15), immediately following ejaculation (time 0), and post-ejaculation periods (times +15, +30, +60).
* Signifies significant increase from time -15 ($P < 0.001$)

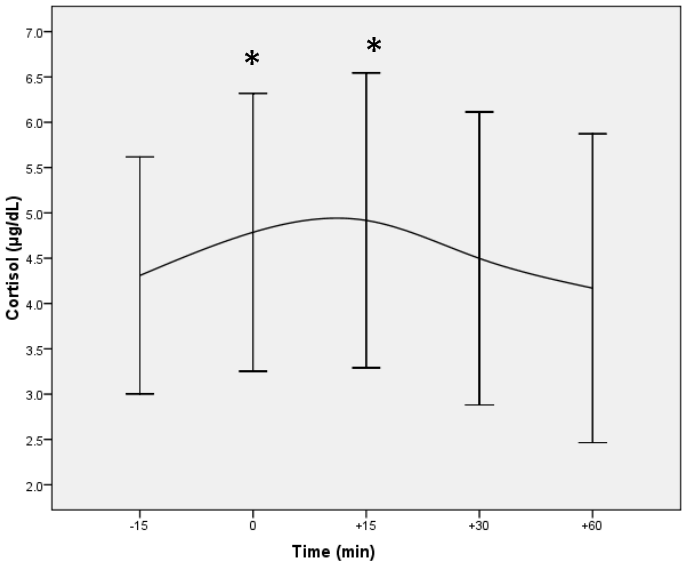

Figure 3. Mean cortisol concentration for all stallions (± SD) for pre-mating (time -15), immediately following ejaculation (time 0), and post-ejaculation periods (times +15, +30, +60).
* Signifies significant increase from time -15 ($P < 0.05$)

Table 1. Correlation coefficients across all data for libido scores and testosterone (T), estrone sulphate (ES), cortisol (C), and ratio of estrone sulphate to testosterone (ES/T)

Time	T	ES	C	ES/T
-15	0.149	-0.418 *	0.377*	-0.402 *
0	0.212	-0.079	0.355*	-0.318
+15	0.093	-0.224	0.448 *	-0.211
+30	0.246	-0.339*	0.323	-0.406 *
+60	0.178	-0.415 *	0.246	-0.386 *

* signifies significant correlation at $P < 0.05$

Table 2. Correlation coefficients across all data for reaction time and testosterone (T), estrone sulphate (ES), cortisol (C) and ratio of estrone sulphate to testosterone (ES/T)

Time	T	ES	C	ES/T
-15	0.021	0.407 *	-0.325	0.246
0	-0.064	0.029	-0.199	0.117
+15	-0.002	0.085	-0.321	0.048
+30	-0.073	0.201	-0.270	0.284
+60	-0.065	0.341*	-0.262	0.329

* signifies significant correlation at $P < 0.05$

Table 3. Correlation coefficients across all data for semen parameters and testosterone (T), estrone sulphate (ES), cortisol (C), and ratio of estrone sulphate to testosterone (ES/T) at time -15

Hormone	Conc. (x 10^6)	Vol. (ml)	PM (%)
T	-0.086	0.203	0.400*
ES	0.148	0.034	0.086
C	-0.283	0.195	0.200
ES/T	0.182	-0.166	-0.347*

* signifies significant correlation at $P < 0.05$

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METABOLIC AND PHYSICAL EFFECTS OF PSYLLIUM SUPPLEMENTATION ON QUARTER HORSES

J. L. Peterson, S. J. Moreaux, J. G. P. Bowman, P. G. Hatfield, J. G. Berardinelli, and J. Olsen
Montana State University, Bozeman

ABSTRACT: Eight 11- to 16-yr-old Quarter Horses were used in a completely randomized design to determine the effects of psyllium supplementation on BW, tailhead fat, neck circumference, plasma glucose, serum insulin, and plasma leptin levels. Horses were stratified by sex and BW, and allowed a 12-d adaptation period to hay and grain. During the following 42 d, horses were individually fed a mixed grain (corn, oats, barley and molasses) ration at 0.5% BW, grass/alfalfa hay at 1.5% BW, and 1 of 2 treatment supplements. Supplements were: 1) 90 g/d psyllium or 2) an isocaloric control supplement. Horses were housed individually in 4 x 7 m pens, with free access to water. Indwelling jugular catheters were placed and blood was collected every 30 min for 6 h beginning at 0730 on d 42. Glucose analysis was performed using a spectrophotometric method and insulin concentrations were measured with an RIA kit validated for horse blood. Blood glucose and serum insulin levels were analyzed using repeated measures. Plasma leptin, tailhead fat, BW, and neck circumference were analyzed using analysis of variance. No treatment effects or treatment by time interactions were found for blood glucose ($P \geq 0.34$). There was a treatment by time interaction ($P = 0.09$) for insulin. Serum insulin levels tended to be higher ($P = 0.10$) for psyllium-supplemented horses compared to non-supplemented horses at 120 min post feeding. Treatment had no effect ($P > 0.35$) on serum insulin concentration at any other time. Leptin concentration did not differ ($P \geq 0.53$) between psyllium-supplemented and control supplemented horses. Change in BW and tailhead fat did not differ ($P \geq 0.12$) between treatments. Neck circumference increased 1.7 cm more ($P = 0.01$) for psyllium-supplemented horses compared to control-supplemented horses. Psyllium supplementation increased neck circumference and serum insulin concentration 120 min after feeding in Quarter Horses.

Key Words: glucose, horse, insulin, psyllium

Introduction

Psyllium is commonly used by veterinarians and horse owners as a treatment or preventative for sand colic. The psyllium seed husk forms a gel-like substance in the gastrointestinal tract that helps the horse pass ingested sand and dirt which becomes entrapped in the large colon. Research supports the use of psyllium supplementation for the treatment and prevention of sand colic, (Hotwagner and Iben, 2007; Landes et al., 2008) but the effects of psyllium on horses' metabolism and physical characteristics have not been studied. In humans, however, effects of psyllium supplementation have been documented (Sierra et al., 2001; Sierra et al., 2002). Research conducted on normal healthy humans reported that psyllium supplementation increased insulin sensitivity, lowered blood glucose concentrations, and reduced body weight (Sierra et al., 2001). Obesity and insulin resistance have become major health concerns in the equine industry primarily due to their link with the development of laminitis (Frank et al., 2006). If horses react to psyllium in the same manner as humans, psyllium could possibly function as a preventative for laminitis by decreasing weight and increasing insulin sensitivity. Based on the research conducted in humans, we hypothesized that psyllium supplementation in horses would increase insulin sensitivity, lower blood glucose concentrations, and reduce BW. Therefore, the objective of this study was to evaluate the effects of psyllium on BW, neck circumference, tailhead fat, plasma glucose, serum insulin, and plasma leptin concentrations in Quarter Horses.

Materials and methods

Animals. Procedures were approved by Montana State University's Agricultural Animal Care and Use Committee. Eight (4 mares and 4 geldings) 11- to 16-yr-old Quarter Horses (535.8 ± 25.3 kg initial BW) with BCS of 5 or 6 out of 9 (Henneke et al., 1983), were used in this study. All horses had the same sire and were selected from the Montana State University equitation herd. Horses were individually limit-fed a ration of mixed hay and grain. The feeding regimen met or exceeded energy requirements described by the NRC guidelines for mature horses at maintenance (NRC, 2007). The diet consisted of hay and grain, fed in 2 equal portions at 0800 and 1600. Horses were housed in individual 4 x 7 m pens and allowed *ad libitum* access to clean water and a plain white salt block. All feed was consumed by every horse each day throughout the study.

Design and Treatment. Horses were first stratified by sex and BW, randomly assigned to 1 of 2 treatment groups, and then allowed a 12-d adaptation period to hay and grain (Table 1). During the following 42 d supplementation period, horses were individually fed a mixed grain (corn, oats, barley and molasses) ration at 0.5% BW, grass/alfalfa hay at 1.5% BW, and 1 of 2 treatment supplements. Supplements were: 1) 90 g/d psyllium (psyllium-supplemented) or 2) an iso caloric grain based control supplement (control-supplemented). The amount of psyllium fed was extrapolated from the dose given to human patients in a similar study by Sierra et al. (2002) and was consistent with the Psyllium EQ® label recommendations for prevention of sand colic in horses.

Measurements and Collections. Measurements of BW, tailhead fat, neck circumference, and plasma leptin were taken on d 0 and 42. Blood samples were collected for determination of plasma glucose and serum insulin concentrations on d 42. For blood collections, indwelling jugular catheters were placed at least 2 h prior to the first blood draw which began at 0730 (30 min before feeding) and continued every 30 min for 6 h. Horses were kept in individual pens and left undisturbed with the exception of blood collection. The feed used during blood collection was the same treatment protocol described in the design and treatment section and each horse consumed all of its feed.

Analytical Methods. Body weight was measured with an electronic scale (Tru Test AG 500). Tailhead fat thickness was measured ultrasonically by a multi-frequency linear array transducer (Sonovet 600) 10 cm lateral to the sacral spinous processes and 11 cm cranial to the tailhead origin (Kane et al., 1987). Mean neck circumference was calculated using 3 equidistant measurements between the poll and withers with horses restrained in stocks and the head held in a normal upright position (Frank et al., 2006).

Blood samples were collected into vacutainers containing potassium oxalate and sodium fluoride for analysis of plasma glucose and leptin, and into vacutainers without additives for analysis of serum insulin. Within 1 min of collection, vacutainers were placed on ice. Samples were centrifuged (1,600 x *g*) at 4 ºC, and plasma and serum were decanted and stored at -20ºC. Glucose analysis was performed in duplicate using a spectrophotometric method based on glucose hexokinase (Glucose hexokinase kit; Sigma Diagnostics, St. Louis, MO). The intra- and interassay CV were 7.7 and 8.4%, respectively, for a pool of plasma that contained a mean of 113.6 mg/dL glucose. The low pool plasma contained a mean of 36.3 mg/dL and the intra- and interassay CV were 6.6 and 12.8%, respectively. The sensitivity of the assay for glucose was 20 mg/dL. Serum insulin was determined in duplicate by use of a commercially available radioimmunoassay (Insulin kit; Coat-a-Count Diagnostics, Los Angeles, CA) validated for horse blood (Freestone et al., 1992). The intra- and interassay CV were 20 and 22%, respectively, for serum insulin concentrations of 16 µIU/mL, and limit of detection was 3.3 µIU/mL. Plasma leptin concentrations were determined using a commercially available mulit-species radioimmunoassay (Multi-Species Leptin RIA kit; Linco Research, St. Charles, MO) that had been validated for measuring leptin in equine serum and plasma (Fitzgerald and McManus, 2000). Samples were run in duplicate. The intra-assay CV for leptin high and low pools were 0.76 and 0.31%, respectively.

Hay, grain, and psyllium were analyzed for nutrient composition (O.O. Thomas Nutrition Center, Bozeman, MT). Starch and sugar content were also analyzed (Equi-Analytical Laboratories, Ithaca, NY). Sugar content was analyzed as water soluble carbohydrate and ethanol soluble carbohydrate. Non-structural carbohydrate for the hay was calculated with the following equation:

NSC= 100 - CP - NDF - crude fat - ash

Digestible energy was calculated with the following equations (NRC, 2007):

For hay: DE, Mcal/kg = 4.22- 0.11(%ADF) + 0.0332(%CP) + 0.00112(%ADF^2)
For grain and psyllium: DE, Mcal/kg = 4.07 - 0.055(%ADF)

Analyzed and calculated nutrient composition of feeds are presented in Table 1.

Statistical Analyses. Each horse was considered an experimental unit. Plasma glucose and serum insulin concentrations were analyzed using a mixed model with repeated measures with treatment, time, and the interaction of treatment and time as fixed effects, horse as random effect, and compound symmetry as the covariance structure (SAS Inst. Inc., Cary, NC). Areas under the concentration-time curve were calculated by the trapezoidal method (Gibaldi and Perrier, 1982) for plasma glucose and serum insulin and were analyzed by ANOVA of SAS. Changes in plasma leptin concentrations, neck circumference, BW, and tailhead fat between d 0 and 42 were also analyzed using ANOVA of SAS. Least square means were separated using the LSD method when $P < 0.10$.

Results

The change in leptin concentration between d 0 and 42 was not different for psyllium-supplemented and control-supplemented horses (Table 2). Change in BW and tailhead fat did not differ between treatments (Table 2). Neck circumference increased from d 0 to d 42 in both psyllium- and control-supplemented horses, however, there was a greater increase ($P = 0.01$) in neck circumference of psyllium-supplemented horses than in non-supplemented horses (Table 2). There was no treatment or treatment by time interaction for glucose concentrations (Figure 1). Although postprandial insulin concentrations did not differ between treatments, there was a treatment by time interaction ($P = 0.09$; Figure 2). This appeared to be caused by an increase in insulin concentrations from 60-120 min after feeding in psyllium-supplemented horses, whereas, insulin concentrations decreased during this time in control-supplemented horses. Insulin concentration tended to be greater ($P = 0.10$) at 120 min after feeding for psyllium-supplemented compared with control-supplemented horses (Figure 2). Areas under the concentration-time curves for glucose and insulin did not differ between psyllium-supplemented and control-supplemented horses (Table 3).

Discussion

It was our hypothesis that horses would respond to psyllium supplementation much like humans do. However, after 42 d of administering psyllium to horses, the effects of the supplement were different than those reported in similar studies performed on humans. Other investigators (Jose-Cunilleras et al., 2004) equated area under the curve of glucose concentration to a relative glycemic index of the diet. Our study demonstrated a delay in the peak of insulin

in psyllium-supplemented horses but no difference between treatments in cumulative area under the curve. Insulin peaked at a greater concentration in psyllium-supplemented horses compared to non-supplemented horses. It may be that psyllium slowed the passage rate of ingesta through the small intestine and delayed glucose absorption and the resulting insulin response. The dose of psyllium used in this study was similar to that which is recommended for the prevention of sand impaction in horses. Daily supplementation of horses with psyllium at this level does not appear to alter the glycemic index of the diet to a degree that would require any adjustment in the energy content. Likewise, psyllium supplementation at the rate administered in this study would not appear to be therapeutic for obesity or insulin resistance in horses as it is in humans.

Implications

The results of this study indicate that psyllium fed daily at a dose of 90 g may not affect the glycemic index of a diet, but may slow passage rate of ingesta through the small intestine and delay glucose absorption in horses. It does not appear that psyllium supplementation lowers blood glucose or increases insulin sensitivity. Furthermore, it does not seem to reduce weight in horses as reported in humans. Therefore, pysllium may not be a reasonable treatment for obesity or insulin resistance in horses. Further research with varying diets and dosages of psyllium are warranted to determine alternative therapeutic effects of psyllium on horses.

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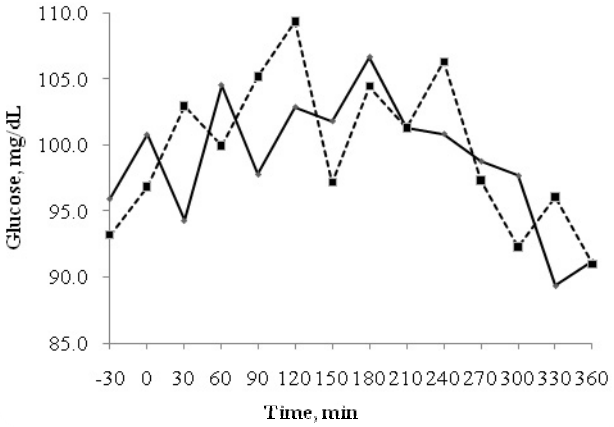

Figure1. Least square means for plasma glucose concentrations of psyllium-supplemented (solid line) and control-supplemented (dashed line) horses. Effects of treatment and treatment x time ($P \geq 0.34$). SEM = 7.02.

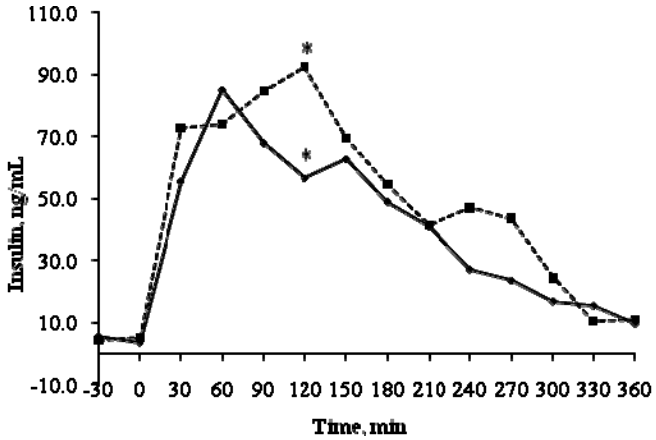

Figure2. Least square means for serum insulin concentrations of psyllium-supplemented (solid line) and control-supplemented (dashed line) horses. Effect of treatment x time ($P = 0.09$). SEM = 21.85. Asterisks indicate a difference between means at 120 min ($P = 0.10$).

Table 1. Chemical composition (DM basis) of hay (grass/alfalfa mix), grain (corn, oat, barley and molasses), and psyllium (psyllium seed husk) fed to horses

Item	Hay	Grain	Psyllium
DM, %	94.4	94.1	94.0
CP, %	11.1	14.8	12.5
ADF, %	25.9	8.1	11.4
NDF, %	55.2	-	-
Starch, %	2.0	44.8	17.2
Sugars, %			
Water soluble	7.8	4.4	7.2
Ethanol soluble	2.0	3.0	2.2
NSC,[a] %	28.8	-	-
Crude fat, %	0.9	3.9	3.7
Ash, %	8.9	7.5	7.3
DE, Mcal/kg	2.6[b]	3.9[c]	3.7[c]

[a]Nonstructural carbohydrate (NSC = 100 – CP – NDF – crude fat – ash).
[b] DE, Mcal/kg = 4.22 – 0.11(%ADF) + 0.0332(%CP) + 0.00112(%ADF^2); NRC, 2007.
[c] DE, Mcal/kg = 4.07 – 0.055(%ADF); NRC, 2007.

Table 2. Changes (d 0 to 42) in plasma leptin concentration, BW, neck circumference, and tail-head fat for psyllium-supplemented and control-supplemented mature Quarter Horses[1]

Variable	Control-supplemented	Psyllium-supplemented	SEM	*P*-value
n	4	4		
Leptin, ng/mL	-0.1	-0.4	1.20	0.74
BW, kg	19.2	27.0	13.48	0.12
Neck circumference, cm	1.1	2.8	0.27	0.01
Tail-head fat, mm	1.9	2.4	1.95	0.73

[1] Changes in these variables were calculated as the difference between measurements taken on d 0 and 42.

Table 3. Area under the curve (AUC)[1] for plasma glucose and serum insulin over 6.5-h sampling period in control-supplemented and psyllium-supplemented mature Quarter Horses

Variable	Control-Supplemented	Psyllium-Supplemented	SEM	*P*-value
n	4	4		
Glucose AUC, mg·dL^{-1}·min^{-1}	53.2	53.6	2.21	0.82
Insulin AUC, ng·mL^{-1}·min^{-1}	20.0	24.5	7.12	0.41

[1] Cumulative area of 14 samples (collected every 30 min for 390 min) calculated by the trapezoidal method (Gibaldi and Perrier, 1982).

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THE EFFECTS OF MATERNAL OBESITY AND HIGH NUTRIENT INTAKE ON OVINE COLOSTRUM NUTRIENT COMPOSITION

N. M. Long[*§], A. B. Uthlaut[*§], D. C. Rule[§], P. W. Nathanielsz[*†], and S. P. Ford[*§]

[*]Center for the Study of Fetal Programming, [§]Department of Animal Science, University of Wyoming, Laramie, [†]Department of Obstetrics and Gynecology, University of Texas Health Sciences Center, San Antonio.

Abstract: Multiparous whiteface ewes were stratified by parity, BW, and BCS and allocated to control (C; 100 % of NRC recommendations, n = 9), or obese (Ob, 150 % of NRC, n = 9) groups from 60 d before conception through parturition. A colostrum sample (~ 40 ml) was collected within 6 h of lambing and stored at -20 ° C, until evaluated for DM, protein (Leco Nitrogen Analyzer) and fatty acid (FA) composition (GLC). Data was analyzed as a randomized complete block with treatment, birth type (Twin or single), and their interaction in the model. Colostrum DM was unaffected ($P > 0.15$) by treatment or birth type and averaged 39.6 ± 3.0 %. Protein content of colostrum had a treatment x parity interaction ($P < 0.01$) with colostrum from Ob ewes (n = 7) producing twins having decreased protein concentration compared with C ewes producing singletons(n = 6), C ewes producing twins (n = 5), and Ob ewes producing singletons (n = 4;32 ± 3% vs. 56 ± 2, 58 ± 4, and 51± 3%, respectively). Total FA concentration in colostrum was increased ($P = 0.04$) in Ob ewes compared with C ewes (491 ± 36 vs. 370 ± 36 mg/m DM). Further, singleton birth resulted in decreased ($P = 0.02$) total FA in colostrum when compared with twin birth (362 ± 33 vs. 199 ± 40 mg/g DM). Concentrations of individual FA in colostrum were increased on Ob compared with C ewes ($P \leq 0.04$, Table 2). Thus, maternal obesity resulted in decreased colostrum protein concentrations in twinning ewes and increased total FA concentrations regardless of birth type. These data indicate that maternal obesity can impact postnatal nutrition of offspring.

Keywords: Colostrum, Fatty acid composition, Maternal obesity

Introduction

Fat content of colostrum is important for energy supply, glucose homeostasis and proper thermoregulation in the newborn (Pettigrew, 1981, Le Dividich et al., 1994, 1994). Increased colostrum fat due to supplementation of cows with safflower oil prepartum allowed newborn calves to be better able to maintain body temperature when housed at 0 ° C (Quigley and Drewry, 1998). Protein content of colostrum is also important to the newborn for protein synthesis. Protein synthesis for newborn lambs is estimated at 1.4 g/h per kg of BW (Yvon et al., 1993). The availability of amino acids is also important for gluconeogenesis (Quigley and Drewry, 1998). Ewes BCS does not appear to influence the fat or protein content of colostrum (Thomas et al., 1988). However, this study looked atewes whose BCS did not exceed 3.5 on a 1 to 5 BCS scale therefore the effects of extreme obese has not been evaluated. A reduced feed intake to 70 % of ME requirements during late gestation in the ewe did not alter colostrum fat content but did tend to reduce colostrum protein (Banchero et al., 2006).

Maternal obesity and overnutrition during gestation decreased the colostrum yield after parturition in the ewe (Wallace et al., 2005, Swanson et al., 2008). However, maternal obesity and high nutrient intake, or nutrient restriction during gestation did not affect percent butterfat, protein or lactose of colostrum (Swanson et al., 2008). Obese rats have higher than normal fat concentrations in milk , but the volume of milk produced is substantially reduced (Rasmussen et al., 2001). Pregnant rats fed a high-fat diet before lactogenesis have difficulty initiating lactation, and during lactation, they exhibit low milk production which is associated with poor pup growth and high pup mortality rates (Rolls and Rowe, 1982).

We hypothesize that maternal obesity and high nutrient intake before and throughout gestation will alter the protein content and total and specific FA composition of colostrum in the ewe.

Materials and Methods

This study was conducted at the University of Wyoming and all procedures were approved by the University of Wyoming Animal Care and Use Committee. Multiparous Rambouillet X Columbia crossbred ewes were stratified by parity, BW and BCS and allotted to one of two treatments. Control (**C**, n = 10) ewes were fed 100 % of NRC (1985) requirements from 60 d before conception and throughout gestation, while obese (**Ob**, n = 11) ewes were fed 150 % of NRC requirements over the same period. Ration composition is given in Table 1. Ewes were mated to 1 of 3 Columbia rams that were rotated between groups every 7 d. Ewe BW was recorded at the start of the experiment and BW and BCS (1 to 9 scale; Sanson et al., 1993) were assigned at the start of breeding, then 60 d later, and again in late gestation (~d 135 of gestation). Ewes were allowed to lamb naturally and a sample of colostrum was collected within 6 hrs of lambing and stored at -20 ° C until analyzed.

Colostrum DM was determined on 0.3 g samples of colostrum by AOAC (1990). Colostrum was then freeze dried (model 10-MR-TR Freeze Dryer, VirTis Co., New York). Protein concentration was determined in 0.1 g of freeze dried colostrum samples by Leco Nitrogen Determinator (model FP-528; Leco Corp., St. Joseph, MI) in accordance with AOAC (1990). Fatty acid composition was determined by GLC as outlined by Moutsioulis et al (2008).

Briefly, 1 mL of a 15-mg/mL internal standard solution of 13:0 (glyceryl-tritridecanoate, Sigma Chemical Co, St. Louis, Mo) in chloroform was dried under nitrogen, and a 100-mg sample of freeze-dried colostrum was added. Fatty acid methyl esters (FAME) were prepared using direct transesterification with 0.2 N KOH in Methanol (Murrieta et al., 2003) . Fatty acid methyl esters were separated using an Agilent 6890 GLC (Agilent Technologies, Inc, Palo Alto, Calif) equipped with a 100 m × 0.25 mm fused silica capillary column (SP-2560, 0.2 μm film thickness, Supelco, Bellefonte, Pa) and flame ionization detector. Fatty acid methyl ester peaks were identified by comparing retention times with FAME standards (Nu-Check Prep, Inc, Elysian, MN, and Matreya, Inc, Pleasant Gap, Pa). FAME were evaluated using ChemStation software (Agilent Technologies, Inc). All samples were analyzed in duplicate. Total FA and specific fatty acid concentrations were determined using calculations from Murrieta et al (2003).

Data for colostrum DM, CP, total and specific FA were analyzed as a randomized complete block with treatment, birth type (singleton or twin) and their interaction in the model using the GLM procedure of SAS ((SAS Inst. Inc., Cary, NC). Results are presented as least square means ± SEM for treatment and birth type since there was no interaction ($P > 0.15$) other than for colostrum protein. Significance was considered at $P \leq 0.05$ and a tendency was considered at $P \leq 0.1$.

Results

Ewe BW at the beginning of the experiment was similar ($P = 0.58$) between groups and averaged 70.7 ± 1.8 kg. At breeding Ob ewes BW and BCS were increased ($P < 0.0003$) compared with C ewes (84.6 ± 2.6 kg and 6.7 ± 0.2 vs. 69.7 ± 2.8kg and 5.2 ± 0.2, respectively). By 60 d after the start of breeding Ob ewes were still heavier and exhibited a greater BCS ($P < 0.0001$) compared with C ewes (99 ± 3.1 kg and 7.5 ± 0.1 vs. 72.3 ± 0.1 kg and 5.7 ± 0.1, respectively). In late gestation Ob ewes were heavier and exhibited a greater BCS ($P = .0004$) compared with C ewes (114.7 ± 3.9 kg and 7.7 ± 0.2 BCS vs. 90.8 ± 4.3 kg and 6.3 ± 0.3 BCS, respectively).

Colostrum DM % was unaffected by treatment or birthtype ($P = 0.87$ and $P > 0.15$ respectively). Protein content of colostrum had a treatment x parity interaction ($P < 0.01$) with colostrum from Ob ewes producing twins having decreased protein concentration compared with C ewes producing singletons, C ewes producing twins, or Ob ewes producing singletons (32 ± 3% vs. 56 ± 2; 58 ± 4; 51± 3 %, respectively).

Total FAs were increased ($P = 0.04$) in the colostrum of Ob ewes compared with C ewes (491.5 ± 36.2 vs. 370.4 ± 36.2 mg/ g of DM). Total FAs were also increased ($P = 0.02$) in ewes that gave birth to twins compared to ewes that gave birth to singleton offspring (499.0 ± 39.9 vs, 362.9 ± 32.9 mg/g DM, respectively). The FAs 17:0, 18:0, 18: 1 *trans* 11 and 18:2 n-6 were increased ($P < 0.05$, Table 2) as a % of total FA in colostrum from Ob ewes compared with colostrum from C ewes. The FA 10:0 tended ($P = 0.09$, Table 2) to be increased as a % of total FA in colostrum from Ob ewes compared with colostrum from C ewes. In contrast, 14:0 and 16:0, were increased ($P < 0.05$, Table 2) as a % of total FA in colostrum of C ewes compared with colostrum from Ob ewes. Birth type also altered the composition of specific FAs. The % of total FAs composed of 14:0, 15:0, 16:0, and 16: 1 were increased ($P < 0.04$) in ewes that gave birth to singletons compared to ewes that gave birth to twins (13.61 ± 0.42, 1.30 ± 0.13, 31.98 ± 1.47, and 2.05 ± 0.18 vs. 7.34 ± 0.51, 0.71 ± 0.16, 23.76 ± 1.78, and 1.35 ± 0.22 %, respectively). However, for longer chain FA the opposite was true, with ewes giving birth to twins having greater ($P < 0.003$) quantities of 17:0, 17:1, 18:0, and 18:1 n-9 as a % of total FAs compared with ewes giving birth to singleton lambs (1.98 ± 0.16, 1.26 ± 0.09, 8.00 ± 0.52, and 34.27 ± 2.03 vs. 1.16 ± 0.13, 0.78 ± 0.08, 4.16 ± 0.43, and 20.60 ± 1.68 %, respectively).

The concentration (mg/g DM) of 10:0, 15:1, 17:0, 17:1, 18:0, 18:1 *trans* 11, 18:1 n-9, and 18:2 n-6 increased ($P < 0.05$) and 12:0, CLA, and 20:4 n-6 tended($P < 0.09$) to be increased in colostrum from Ob ewes compared to colostrum from C ewes. Concentrations of 14:0 were increased ($P = 0.03$) in ewes giving birth to singletons compared to ewes giving birth to twins (48.07 ± 3.42 vs. 34.07 ± 4.15 mg/g DM, respectively). However, concentrations of 17:0, 17:1, 18:0, 18:1 n-9, and 18:2 n-6 were increased ($P < 0.03$) in colostrum from ewes that gave birth to twins compared to colostrum from ewes that gave birth to singletons (10.32 ± 1.17, 6.35 ± 0.59, 42.21 ± 452, 174.41 ± 17.93 and 15.16 ± 1.83 vs. 4.46 ± 0.97, 2.98 ± 0.49, 15.91 ± 3.73, 78.278 ± 14.81, and 9.03 ± 1.51 mg/g DM, respectively).

Discussion

This is the first study in the sheep that the authors are aware of which looks at colostrum nutrient and FA composition and how it is affected by maternal obesity and high nutrient intake. Using the same experimental paradigm, we have previously reported that maternal and fetal blood FA profiles were altered by maternal obesity at midgestation and this was associated with increased cotyledon FA transporter 1 and 4 protein (Zhu et al., 2008). If lactating cows are supplemented with soybean oil milk fat was increased and the proportion of FA and FA concentrations were altered (Steele et al., 1971) indicating that circulating FA in the maternal blood could impact FA concentration in milk and therefore colostrum. We have also demonstrated that blood glucose and insulin concentrations were elevated at mid gestation in fetuses gestated by Ob ewes compared with fetuses gestated by C ewes (Zhu et al., 2009) and that glucose was increased and insulin was decreased in the lambs at birth (Ford et al., 2009). Noble et al (1971) showed that alterations in the FA profile of the diet results in alterations in the plasma FA for the first 8 d of life in lambs. This indicated that colostrum FA composition can alter the lambs circulating plasma FA and provide a mechanism for postnatal effects of maternal obesity and high nutrient intake. However, the yield of colostrum has been shown to be reduced in Ob ewes (Wallace et al., 2005, Swanson et al., 2008) and this reduction in yield of colostrum could act to buffer the effects of altered colostrum FA composition on the newborn lamb.

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Table 1. Diet composition

Ingredients, %	
Ground Bromegrass hay [1]	14.02
Ground corn	63.89
Soybean meal	13.33
Liquid molasses	5.66
Limestone	2.24
Ammonium chloride	0.5
Mineralized salt [2]	0.24
Magnesium ckloride	0.1
ADE premix [3]	0.1
Rumensin 80	0.02
Analyzed composition [4]	
DM, %	88.54
NDF, % of DM	24.09
ADF, % of DM	9.99
CP, % of DM	17.39
IVDMD, %	93.92

[1] Mean particle length = 2.54 cm
[2] Contains 13 % NaCl, 10 % Ca, 10 % P, 2 % K, 1.5 & Mg, 0.28% Fe, 0.27% Zn, 0.12 % Mn, 0.01% I, 35 ppm Se, and 20 ppm Co
[3] Contains 110,000 Iu/kg vitamin A, 27,500 IU/kg vitamin D and 660 IU/kg vitamin E
[4] determined by near infrared reflectance spectroscopy

Table 2. Percentages of total fatty acids and concentrations of specific fatty acids in colostrum from obese or control ewes

	% of Total FA		Concentration mg/g DM	
	Obese ewes	Control ewes	Obese ewes	Control ewes
10:0	3.44 ± 0.27^{c}	2.73 ± 0.27^{d}	15.79 ± 1.05^{a}	10.25 ± 1.05^{b}
12:0	2.84 ± 0.25	2.67 ± 0.25	13.97 ± 1.48^{c}	9.83 ± 1.48^{d}
14:0	9.23 ± 0.46^{a}	11.72 ± 0.46^{b}	41.29 ± 3.77	40.84 ± 3.77
15:0	0.99 ± 0.14	1.03 ± 0.14	4.36 ± 0.60	3.59 ± 0.60
15:1	0.14 ± 0.02	0.12 ± 0.02	0.69 ± 0.10^{a}	0.46 ± 0.10^{b}
16:0	25.77 ± 1.62^{c}	29.89 ± 1.62^{d}	122.22 ± 9.73	106.59 ± 9.73
16:1	1.53 ± 0.20	1.87 ± 0.20	7.14 ± 0.76	6.55 ± 0.76
17:0	1.80 ± 0.15^{a}	1.34 ± 0.15^{b}	9.46 ± 1.07^{a}	5.32 ± 1.07^{b}
17:1	1.11 ± 0.08	0.93 ± 0.08	5.71 ± 0.54^{a}	3.62 ± 0.52^{b}
18:0	6.93 ± 0.47^{a}	5.24 ± 0.47^{b}	37.04 ± 4.10^{a}	21.08 ± 4.10^{b}
18:1 trans 11	4.44 ± 0.61^{a}	2.33 ± 0.61^{b}	21.24 ± 2.92^{a}	9.13 ± 2.92^{b}
18:1 n-9	28.18 ± 1.85	26.70 ± 1.85	148.27 ± 16.29^{a}	104.40 ± 16.29^{b}
18:2 n-6	3.03 ± 0.21^{a}	2.32 ± 0.21^{b}	15.26 ± 1.67^{a}	8.93 ± 1.67^{b}
CLA	0.73 ± 0.09	0.61 ± 0.09	3.52 ± 0.54^{c}	2.31 ± 0.54^{d}
20:4 n-6	0.41 ± 0.04	0.42 ± 0.04	1.96 ± 0.20^{c}	1.54 ± 0.20^{d}

[a,b] means differ $P \leq 0.05$
[c,d] means differ $P \leq 0.10$

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FEEDLOT PERFORMANCE, CARCASS CHARACTERISTICS, AND FATTY ACID COMPOSITION OF TISSUES FROM EWE LAMBS FED WHOLE CAMELINA SEEDS

P. L. Price, V. Nayigihugu, W. J. Means, D. C. Rule and B. W. Hess
Department of Animal Science, University of Wyoming, Laramie, 82071

ABSTRACT: Twelve ewe lambs (52.1 ± 3.3 kg BW) were used to determine the effects of feeding whole camelina seeds on feedlot performance, carcass characteristics, and fatty acid composition of the *semitendinosus* muscle (ST), LM, KPH, and adipose tissue collected from the tail head (TH). Six weeks before slaughter, lambs were fed either a low-fat diet consisting of 65.2% corn, 18.0% bromegrass hay, and 15.0% soybean meal (Control, as-fed basis), or whole camelina seeds (14.9% of diet, as-fed basis), which replaced enough of the soybean meal in the Control diet to provide 4.7% added fatty acid (Camelina). Urea was added to produce isonitrogenous diets. Neither feedlot performance nor carcass characteristics were affected ($P \geq 0.43$) by dietary treatment. Total fatty acids (mg/g of freeze-dried tissue) in LM (P = 0.25), ST ($P = 0.90$), and KPH ($P = 0.92$) did not differ between treatments, but lambs fed Camelina tended to have greater ($P = 0.08$) total fatty acids in TH. Weight percentages of 16:0 ($P = 0.03$) and 18:0 ($P = 0.01$) in KPH and 16:0 in ST ($P = 0.03$) and LM ($P = 0.04$) were greater for lambs fed Control. Weight percentages of 18:1*t*-9 ($P < 0.01$), 18:1*t*-11 ($P < 0.01$), 18:1*c*-11 ($P = 0.01$), and 22:1n-9 ($P < 0.01$) in KPH were greater for lambs fed Camelina. Weight percentage of 18:3n-3 in LM ($P = 0.01$), ST ($P = 0.01$), and TH ($P < 0.01$) was greater for lambs fed Camelina; 18:3n-3 also tended to be greater ($P = 0.06$) in KPH of lambs fed Camelina. Weight percentage of 18:1*c*-9 in TH ($P = 0.04$) and ST ($P = 0.02$) was greater, and tended to be greater ($P = 0.06$) in LM of lambs fed Control. A trend for greater 18:2*c*-9*t*-11 in TH ($P = 0.10$) was noted for lambs fed Camelina. Weight percentage of 20:1n-9 in LM ($P < 0.01$), ST ($P < 0.01$), KPH ($P < 0.01$), and TH ($P < 0.01$) was greater in lambs fed Camelina. Feeding whole Camelina seeds to provide an additional 4.7% dietary fatty acid may be a nutritional strategy to alter fatty acid composition in muscle and adipose tissue of lambs.

Key words: lamb, camelina, fatty acids

Introduction

The growth of the biodiesel industry has prompted crop producers to increase production of oilseeds. Camelina (*camelina sativa*) is an oilseed that is being planted in the high mountain plains region. Camelina is easily grown and drought tolerant, which makes it suitable for cultivation in this region. Due to escalating costs of shipping and limited market opportunities in the high mountain plains region, growers of camelina are interested in alternative uses of the seed. Because it is a rich source of fat and protein (38% crude fat and 27% CP; Lardy, 2008), one potential alternative is to incorporate camelina in the diets fed to livestock. Milk fatty acid composition was altered by feeding camelina to lactating dairy cows (Hurtaud and Peyraud, 2007), but limited research has been conducted with feeding camelina to other ruminant livestock.

Adding fat to the diets of livestock is not uncommon, although caution must be taken as high levels may have negative effects on digestion (Nelson et al., 2001). In a recent study conducted by our laboratory, we reported that lambs fed whole camelina seeds had greater intestinal disappearance of many biohydrogenation intermediates including 18:1*t*-9 and 18:1*t*-11, as well as 18:3n-3 (Price et al., 2008). We are unaware of any studies on feedlot performance, carcass characteristics, and fatty acid composition of lambs fed whole camelina seeds. Our hypothesis was that feeding camelina to finishing lambs will not have negative effects growth performance or carcass characteristics, although fatty acid profile of muscle and adipose tissue will be altered. Objectives were to determine the effects of feeding whole camelina seeds on feedlot performance, carcass characteristics, as well as fatty acid composition of the LM, *semitendinosus* (ST), KPH, and tail head fat (TH) in lambs fed whole camelina seeds.

Materials and Methods

General. All procedures were approved by the University of Wyoming Animal Care and Use Committee. Twelve speckle-face crossbred (Columbia × Hampshire × Rambouillet) ewe lambs (52.1 ± 3.3 kg BW) were selected from the University of Wyoming flock and placed in individual pens. After allowing 1 wk for adaptation, 6 lambs were weighed and randomly assigned to 1 of 2 dietary treatments, 3 lambs per treatment. Two weeks later, the remaining 6 lambs were allowed 1 wk for adaptation, weighed, and assigned to 1 of 2 dietary treatments, 3 lambs per treatment.

Diets and Sampling. The **Control** diet contained no supplemental fat and consisted of ground (2.54 cm) bromegrass hay, cracked corn, soybean meal, molasses, limestone, urea, and salt (Table 1). Camelina was added to replace enough of the soybean meal to provide 4.9% added fatty acids (14% of DM). Dietary treatments were formulated for lambs gaining 250 g/d (NRC, 2007). Urea was added to the Camelina to generate isonitrogenous diets. All lambs were individually fed once daily at 0800 for 5 wk. Feed refusals were collected and weighed before each feeding. For lambs that refused feed, the ration was decreased to allow complete consumption the next day. Daily rations were then increased gradually to allow for *ad libitum* consumption. Grab samples of the total mixed

ration were taken periodically throughout the feeding period.

Both groups of lambs were slaughtered after each respective 5 wk feeding period. For both slaughter groups, lambs were fasted for 12 h before transported to the University of Wyoming Meat Laboratory where BW was determined immediately prior to slaughter. Samples of KPH and TH fat for FA analysis were collected on the slaughter floor. Other than HCW, carcass measurements were collected after an overnight chill. Subcutaneous fat thickness at the 12th rib ½ the distance from the medial to lateral edge of the LM, body wall thickness 13 cm from the midline, and 12th rib LM area were measured. These data were collected on both sides and averaged. Areas of the LM were estimated using a 20 dots/inch^2 pork grid (Iowa State University), which was then converted to cm^2. Yield grade, boneless retail cut percentage, and final quality grade were estimated using USDA lamb grading standards (USDA-AMS, 1992) and methods described by Boggs et al. (2006). The LM and ST muscles were dissected and placed in air tight bags for subsequent FA analysis. During this time, 4 of the LM and ST samples (3 Control; 1 Camelina) from the second slaughter group were misplaced before analysis.

Table 1. Ingredient compostion of diets fed to lambs

	Dietary treatment[1]	
	Control	Camelina
Ingredients, % of DM		
Bromegrass hay	17.38	16.37
Cracked corn	61.12	61.12
Soybean meal	14.46	0.13
Camelina	-	14.46
Salt	1.05	1.05
Urea	-	1.05
Limestone	1.88	1.88
Molasses	4.10	4.10

[1]Camelina was added so that the diet contained 4.7% added fatty acids (DM-basis).

Laboratory Analysis. Feed samples were analyzed for DM (AOAC, 1990). Muscle samples were trimmed of excess fat, freeze dried (Genesis 25 freeze dryer, The VirTis Co., Gardiner, NY), ground with a coffee grinder (Braun Aromatic, Braun, Woburn, MA), and stored at -20°C before analysis. Muscle and adipose tissue samples were weighed in duplicate, 200-mg of muscle and 30-mg of adipose tissue, and prepared for FA analysis by direct trans-esterification (Murrieta et al., 2003).

Statistical Analysis. Lamb growth performance, carcass characteristics, and FA data were analyzed as a randomized complete block design using the GLM procedure of SAS (Version 9.1, 2002, SAS Inst., Inc., Cary, NC). Date of slaughter served as the blocking factor and animal as the experimental unit.

Results and Discussion

Feedlot Performance and Carcass Characteristics. Initial ($P = 0.58$) and final ($P = 0.43$) BW, ADG ($P = 0.70$), DMI ($P = 0.18$), and G:F ($P = 0.47$) did not differ among dietary treatments (Table 2). Hot carcass weight ($P = 0.68$), LM area ($P = 0.46$), fat thickness covering the 12th rib ($P = 0.61$), yield grade ($P = 0.58$), flank streaking ($P = 0.74$), body wall thickness ($P = 0.94$), conformation score ($P = 1.00$), percentage of boneless retail cuts ($P = 0.97$), and quality grade ($P = 0.47$) did not differ among dietary treatments; and maturity tended ($P = 0.08$) to be greater for lambs fed Camelina. These results are consistent with another report by our laboratory (Bolte et al., 2002) in which no differences in carcass characteristics were observed when lambs were supplemented with 5% additional fat as safflower seeds.

Fatty Acid Compostion. Total fatty acids (mg/g of freeze-dried tissue) in LM ($P = 0.25$), ST ($P = 0.90$), and KPH ($P = 0.92$) did not differ between treatments, but lambs fed Camelina tended to have greater ($P = 0.08$) total fatty acids in TH. Weight percentage of 16:0 in KPH ($P = 0.03$), ST ($P = 0.03$), and LM ($P = 0.04$) was greater for lambs fed Control. Lambs fed supplemental fat from safflower seeds also had a lesser weight percentage of 16:0 in adipose tissue and muscle (Bolte et al., 2002). Weight percentage of 18:0 ($P = 0.01$) in KPH was greater for lambs fed Control. Weight percentages of 18:1*t*-9 ($P < 0.01$), 18:1*t*-11 ($P < 0.01$), and 18:1*c*-11 ($P = 0.01$) in KPH were greater for lambs fed Camelina. This coincides with a previous experiment in our laboratory in which intestinal disappearance of these FA was greater for lambs fed the same Camelina diet (Price et al., 2008). The FA of particular interest because of its potential to promote myocardial lipidosis (Kramer et al., 1990), 22:1n-9 (erucic acid) was greater ($P < 0.01$) in KPH for lambs fed Camelina; however, 22:1n-9 was not detected in TH, LM, or ST of lambs fed Camelina. Weight percentage of 18:3n-3 in LM ($P = 0.01$), ST ($P = 0.01$), and TH ($P < 0.01$) was greater for lambs fed Camelina; 18:3n-3 also tended to be greater ($P = 0.06$) in KPH of lambs fed Camelina. A trend for greater 18:2*c*-9*t*-11 CLA in TH ($P = 0.10$) was noted for lambs fed Camelina. Hurtaud and Peyraud (2007) reported greater weight percentage of 18:3n-3 in milk of dairy cows fed camelina seeds and camelina meal, as well as increased milk content of CLA, particularly 18:2*c*-9*t*-11. Weight percentage of 18:1*c*-9 in TH ($P = 0.04$) and ST ($P = 0.02$) was greater, and tended to be greater ($P = 0.06$) in LM of lambs fed Control. These results were consistent with Bolte et al. (2002), in which lambs not fed supplemental fat had a greater weight percentage of 18:1*c*-9 in muscle and adipose tissue than lambs fed safflower seeds. Weight percentage of 20:1n-9 in LM ($P < 0.01$), ST ($P < 0.01$), KPH ($P < 0.01$), and TH ($P < 0.01$) was greater in lambs fed Camelina. This was expected as 20:1n-9 makes up 16% of the total FA profile of camelina, and was not completely hydrogenated in the rumen (Price et al., 2008).

We conclude that adding Camelina to the diet of finishing lambs had no effect on feedlot performance and carcass characteristics. Only a small amount of 22:1n-9 was found in KPH of lambs fed Camelina. Feeding Camelina also increased weight percentages of 18:3n-3 and 20:1n-9 in LM, ST, TH, and KPH.

Implications

If available and cost effective, Camelina may be an alternative protein and energy supplement for finishing lambs. As a nutritional strategy to alter fatty acid composition in muscle and adipose tissue, Camelina seeds may be included to provide 4.9% additional fatty acids in diets offered to finishing lambs.

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Table 2. Feedlot performance and carcass characteristics of lambs fed whole camelina seeds

	Dietary treatment			F-test
Item	Control	Camelina	SEM[1]	*P*- value
Initial BW, kg	51.4	52.8	1.66	0.58
Final BW, kg	59.4	61.4	1.68	0.43
ADG, kg	0.23	0.25	0.03	0.70
DMI, kg/d	1.34	1.26	0.04	0.18
G:F	0.17	0.20	0.02	0.47
Carcass characteristics				
HCW	35.0	35.6	0.96	0.68
LM area, cm^2	17.7	18.2	0.51	0.46
12^{th} rib fat thickness, mm	6.03	6.55	0.57	0.61
Yield grade	2.8	3.0	0.23	0.58
Flank streak[2]	281.7	271.7	20.70	0.74
Maturity, A	41.7	58.3	6.00	0.08
Body wall thickness, cm	2.17	2.18	0.11	0.94
Conformation score	11.5	11.5	0.42	1.00
Boneless retail cuts, %	46.1	46.1	0.37	0.97
Quality grade[3]	10.6	10.0	0.37	0.47

[1] n = 6/treatment.

[2] Slight = 100, small = 200, moderate = 300.

[3] 8 = average choice, 10 = high choice, 12 = low prime.

Table 3. Fatty acid composition of adipose tissue and muscle for lambs fed whole camelina seeds

Item	Dietary treatment		SEM[1]	F-test *P*-value	Dietary treatment		SEM[1]	F-test *P*-value
	KPH				Tail head			
FA, mg/g	999	998	31.08	0.92	806	892	30.3	0.08
	wt %				wt %			
16:0	22.69	19.94	0.76	0.03	24.36	23.76	0.82	0.62
18:0	33.70	28.70	1.07	0.01	16.02	16.80	1.56	0.73
18:1*t*9	0.49	0.84	0.06	0.002	0.48	0.48	0.08	0.99
18:1*t*10	1.63	0.97	0.83	0.59	0.93	1.54	0.57	0.47
18:1*t*11	2.62	7.58	0.89	0.004	1.76	3.42	0.73	0.14
18:1*t*12	0.43	0.37	0.02	0.07	0.33	0.32	0.03	0.86
18:1*t*13	0.70	0.65	0.04	0.41	0.45	0.51	0.05	0.43
18:1*c*9	23.90	22.06	0.76	0.12	38.43	34.10	1.28	0.04
18:1*c*11	0.82	1.07	0.05	0.01	1.06	1.03	0.08	0.79
18:1*c*12	0.32	0.28	0.02	0.17	0.33	0.31	0.01	0.12
18:2n-6	2.07	2.63	0.45	0.40	2.01	1.81	0.10	0.22
18:3n-3	0.41	1.08	0.23	0.06	0.47	0.67	0.03	0.003
18:2*c*9*t*11	0.37	0.47	0.09	0.45	0.82	0.65	0.70	0.10
20:1n-9	0.00	1.96	0.16	<0.01	0.00	0.85	0.12	<0.01
22:1n-9	0.00	0.21	0.02	<0.01	-	-		
	LM[2]				*Semitendinosus*[2]			
FA, mg/g	136.0	118.4	7.52	0.25	139.4	142.5	21.9	0.90
	wt %				wt %			
16:0	24.92	23.89	0.37	0.04	24.30	23.00	0.38	0.03
18:0	14.76	15.33	1.10	0.66	13.39	14.17	0.71	0.37
18:1*t*9	0.62	0.47	0.18	0.47	0.54	0.34	0.12	0.18
18:1*t*10	0.61	1.14	0.39	0.27	0.52	0.79	0.26	0.40
18:1*t*11	1.00	1.77	0.66	0.34	1.25	2.08	0.59	0.26
18:1*t*12	0.20	0.23	0.01	0.14	0.22	0.24	0.02	0.23
18:1*t*13	0.28	0.31	0.03	0.40	0.36	0.39	0.04	0.57
18:1*c*9	40.27	38.84	0.62	0.09	40.91	39.77	0.32	0.02
18:1*c*11	1.32	1.44	0.09	0.28	1.37	1.38	0.07	0.83
18:1*c*12	0.34	0.40	0.13	0.68	0.32	0.31	0.02	0.64
18:2n-6	4.98	4.95	0.34	0.95	5.04	4.68	0.30	0.33
18:3n-3	0.64	0.96	0.12	0.01	0.71	0.95	0.05	0.01
18:2*c*9*t*11	0.43	0.41	0.02	0.45	0.53	0.49	0.04	0.36
20:1n-9	0.00	0.45	0.02	<0.01	0.00	0.41	0.02	<0.01
22:1n-9	-	-			-	-		
20:4n-6	1.40	1.46	0.11	0.48	1.62	1.50	0.15	0.49

[1]n = 6/treatment.
[2]n =3 for Control and n = 5 for Camelina.

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EFFECTS OF IMPLANTING AND CASTRATION ON CARCASS CHARACTERISTICS OF HAIR LAMBS FED A HIGH-CONCENTRATE DIET

G. Villalobos[1], F. Núñez G[1], H. González-Rios[2], D. Domínguez[1], H.A. Castillo[1], J.A. Ortega[1]and G. Torrescano[2].

[1]Universidad Autónoma de Chihuahua, Chihuahua, México.

[2]Centro de Investigación en Alimentación y Desarrollo A.C. Hermosillo, Sonora, México.

ABSTRACT: Hair sheep production in Mexico is of economic importance, but castration and implanting are not currently used. In order to evaluate the effects of implanting zeranol and castration on carcass characteristics of finished hair lambs, 18 crossbred lambs (Blackbelly, Dorper, Kathadin, and Pelibuey) were randomly assigned to each of 4 treatments (3 pens/treatment; 6 lambs/pen). Treatments included: intact lambs (IL), IL implanted with 12 mg zeranol (ILI), castrated lambs (CL), and CL implanted with 12 mg zeranol (CLI). Length of the study period was 70 d, during which lambs had *ad libitum* access to an 80% concentrate diet (19% CP, 2.81 Mcal ME/ kg DM). Lambs were slaughtered at 40 kg ±2 kg live weight. At 24 h postmortem cold carcass weight (CCW) was measured and cold carcass dressing (CCD) was estimated. Backfat thickness (BFT, mm) and LM area (REA, cm^2) were measured (USDA, 1992). Warner-Bratzler shear force (WBSF) was measured using LM samples (AMSA, 1995). Data were analyzed in a 2X2 factorial arrangement using PROC MIXED (SAS). Factors included sex condition (SC; intact and castrated) and implanted (IMP) and non implanted (NIMP). The model included the fixed effect of factors and their interaction, sire breed (SB), ewe breed (EB), parturition type (PT) and covariable slaughter weight. Means were tested using LSMEANS/PDIFF. CCW was 4.2% higher (≤0.05) in castrated than intact lambs, in IMP it was reduced 3% (≤0.05). CCD was 2% higher (≤0.05) in castrated than in IMP. REA and BFT were affected (≤0.05) by SC. Intact lambs had 12% more REA, and castrated lambs 60% more BFT. SFWB was not affected by factors. It is concluded that lamb sex condition was critical for the significant changes observed in the main carcass characteristics.

Key Words: lamb carcass quality, implants, zeranol, castration

Introduction

In Mexico, lamb consumption is important, but because national production is still low to satisfy the demand, around 40,000 tons are imported each year from different countries. (Sagarpa, 2006). This suggests the need for using technologies that allow us to increase lamb production.

Anabolic implants are used in different species and they improve production efficiency in 15 to 17%. Zeranol implants improved daily gain and feed efficiency in ram and wether lambs (Nold *et al.*, 1992).

Ram lambs are superior to wethers in lean carcass, growth rate, and feed efficiency (Seideman et al., 1982).

Because some of the consumers complain about the flavor of intact lambs, castration is used in order to improve animal performance, in addition to modify some carcass and meat sensorial characteristics. In Mexico, practices such as lamb implanting and castration are not yet common among commercial lamb producers, mainly because there is little information on animal performance, meat and carcass characteristics of hair lambs.

The objective of this study was to evaluate the effect of castration and zeranol implanting on carcass characteristics of hair lambs fed a high concentrate diet.

Materials and Methods

The study was conducted at Animal Science Department of the Universidad Autonoma de Chihuahua in Chihuahua, Mexico, 28º 35' north latitude and 106º 04' west longitude.

Animals, Facilities and Diet. There were used seventy two Pelibuey, Blackbelly, Dorper, Katadhin, and Suffolk terminal crossbred hair wether lambs with initial body weight of 21.4 ± 2.3 kg. Lambs were treated for internal and external parasites, vaccinated and vitamins A, D and E were applied and individually ear tagged. All pens had free access to water. They had a diet adaptation period of 10 d. During experimental phase they were fed with the same diet of 20% forage and 80% concentrate (DM basis), formulated to contain 19% CP, 2.81 Mcal ME/kg DM, and to get at least .220 kg of ADG (NRC, 1985; Table 1). Feed was served at 0800 and 1600 h.

Table 1. Experimental Diet composition (DM).

Ingredient	DM (%)
Alfalfa hay	20.0
Corn grain, ground	39.13
Corn dry distillery grain	18.00
Cotton seed meal (36% CP)	15.38
Soybean meal	5.00
Corn gluten meal (60% CP)	1.00
Mineral premix ®	0.50
Salt	0.50
Calcium carbonate	0.49

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Treatments. Lambs were randomly asigned to the following treatments (n=18, 3 pens and 6 lambs per pen): intact lambs (IL); intact lambs and implanted with 12 mg zeranol (ILI); castrated lambs (CL); and castrated lambs implanted with 12 mg zeranol (CLI). Castration and implanting were done during the adaptation period.

Carcass Measurements. Once the animals achieved the slaughter weight (40 kg live body weight), they were slaughtered in the Meat Laboratory at the Animal Science Department. Slaughter weight (SW) was recorded; and 24 h *postmortem* hot and cold carcass weight (HCW and CCW, respectively) was measured. Hot carcass dressing (HCD)

and cold carcass dressing (CCD) were estimated, as well as biological dressing (BD). In order to estimate BD it was necessary to estimate empty body weight (EBW). Backfat thickness (mm; BFT), and rib eye area (cm^2) was evaluated in *Longissimus dorsi* (LD) muscle at the 12^{th} rib USDA (1992).

Warner-Bratzler Shear forcer determination (WBSF). A 4 cm-tick chop was removed from the 12^{th} rib region of the LD and frozen at -20 ºC. Chops were thawed at 2 to 4 ºC and roasted on a electric grill (George Foreman) to an internal temperature of 70 ºC. Cooked chops were then cooled, wrapped in freezer paper, and held overnight in a 2 to 4 ºC cooler before Warner-Bratzler shear force (WBSF) values were determined. Six to eight cores (1.3 cm) were removed from each sample, parallel to the longitudinal orientation of the fiber. Cores were sheared once using the WBS blade attached to Texture Analyzer Machine.

Experimental design and statistical analysis. Data were adjusted to a completely random experimental design in a 2X2 factorial arrangement, including as factors sex condition (SC: intact and castrated) and zeranol implants (IMP, implanted; NIMP, nonimplanted). Analysis of variance was made using PROC MIXED in SAS (SAS, 2001). The adjusted mixed model included the fixed effect for the factors and their interaction, slaughter group, ram breed (RB), ewe breed (EB), parturition type (PT) and as random effect slaughter weight. Variables SB, EB, y PT, were not significant and were eliminated of the model. Means were tested using LSMEANS/PDIFF of SAS (2001)

Results

Significance of adjusted model. Observed significance for each variable based in the adjusted model are presented in Table 2. It stands out the significant effect (P<0.001) of SC in all variables. The factor IMP, had effect (P<0.05) on carcass weight (HCW and CCW), and carcass dressing (HCD, CCD, and BD). HCW (P=0.09), HCD (P=0.07), and REA (P=0.07) tended to be affected by the interaction of these two factors. Average values description and their significance are presented for each experimental group..

Carcass weight (HCW and CCW). Average values for carcass weight are shown in Table 3. HCW as well as CCW were affected (P<0.05) by the principal factors SC and IMP, but not by their interaction. Castrated lamb carcasses (CL + CLI) were 4.2 % heavier than those of intact lambs (IL + ILI). Regarding implantation, carcasses of non implanted animals were 3% heavier than those of implanted lambs.

Carcass dressing (HCD, CCD and BD). Carcass dressing results are presented in Table 3. These were significantly affected by SC and implantation with zeranol. Castrated lambs (CL + CLI) had 2.15% (absolute value) more CCD than intact lambs (IL + ILI).

In regard to interaction, carcasses of treatment CL had 2.9% more dressing (P<0.05) than those of ILI (53.55% vs. 50.65%, respectively). BD of castrated lambs in absolute values was 2.3% higher (P<0.05) than that for intact lambs (58.36% vs 56.09%, respectively). Likewise, non implanted lambs had 1% more BD (P<0.05) compared to implanted lambs.

Rib eye area (REA) and Backfat tickness (BFT). Observed values for REA and BFT were strongly influenced by SC (P<0.001), but not by implants (P>0.05). Intact lambs carcasses had REA 12% higher than castrated lambs. BFT in carcasses of castrated animals was in the levels of 4 mm thick, while in intact animals it was of 2.4 mm, it means 60% more for castrated lambs. A positive correlation (P<0.001) was also observed between slaughter weight and the values for REA and BFT.

WBSF. No effect was found (P>0.05) of SC, IMP nor their interaction on SFWB values of LD. Values of SFWB for all treatments were in the 5 kg force levels, which can be considered of medium tenderness (Shackelford et al., 1999).

Discussion

Carcass dressing results are within the range reported by other researchers for hair lambs (Field *et al.*, 1993; Rubio *et al*., 2004; y Schilling, 2005). In contrast, Lirette *et al.* (1984) did not found differences in carcass dressing between castrated and intact lambs.

Regarding the use of zeranol, Nold *et al.* (1992), and Olivares and Hallford (1990) did not observed differences in carcass dressing between implanted and non implanted animals.

Other studies (Arnold and Meyer., 1988; Maiorano *et al.*, 1993; Nold *et al.*, 1992) report similar results to those found in this study, related to increase REA in intact and BFT in castrated lambs. While Crouse et al. (1981) found differences in REA between intact and castrated.

Regarding the effect of implants on these two variables, results have been in contrast because some (Lough et al., 1993; Olivares and Hallford, 1990) mention that they are not modified by implanting the animals, while Huffstedler et al. (1996) have observed incremented REA by using zeranol, these authors argue that the implant promotes protein deposition in muscle, and that it increases muscular development.

Implications

Sex condition of the lambs was determinant in the changes in major carcass characteristics. Carcasses of castrated lambs had better dressing, higher BFT and hence a better general fatness than that of intact lambs. However, REA was higher in intact lambs.

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Table 2. Probability values obtained in the análisis of variante of the adjusted model for each variable in carcass quality.

Term	HCW	CCW	HCD	CCD	BD	REA	BFT	WBSF
SC	0.0001	0.0001	0.0001	0.001	0.0001	0.0001	0.001	0.0005
IMP	0.008	0.038	0.008	0.046	0.002	0.789	0.070	0.658
SC*IMP	0.090	0.078	0.074	0.050	0.318	0.090	0.423	0.098
SW	0.163	0.165	0.030	0.013	0.100	0.001	0.010	0.356

HCW: hot carcass weight; CCW: cold carcass weight; HCD: hot carcass dressing; CCD: cold carcass dressing; BD: biological dressing; REA: rib eye area; BFT: back fat thickness.

Table 3. LSM (±SE) for carcass characteristics of hair lambs.

	IL	ILI	CL	CLI
Variable				
SW (kg)	41.0±1.01a	41.40± 0.9a	41.13 ±1.01a	39.43 ±1.08a
HCW (kg)	20.7 ± 0.16^{b}	20.1 ± 0.16^{a}	21.31 ± 0.16^{b}	21.23 ± 0.17^{b}
CCW (kg)	19.81 ± 0.16^{a}	19.26 ± 0.15^{a}	20.45 ± 0.16^{b}	20.48 ± 0.17^{b}
HCD (%)	50.7 ± 0.39^{ab}	49.31 ± 0.38^{a}	52.24 ± 0.39^{b}	51.94 ± 0.41^{b}
CCD (%)	52.14 ± 0.38^{ab}	50.65 ± 0.37^{a}	53.55 ± 0.38^{c}	53.55 ± 0.41^{bc}
BD (%)	56.8 ± 0.34^{ab}	55.38 ± 0.32^{a}	58.74 ± 0.33^{c}	57.99 ± 0.35^{bc}
REA (cm^2)	16.48 ± 0.36^{b}	15.72 ± 0.34^{ab}	14.57 ± 0.35^{a}	15.01 ± 0.37^{ab}
BFT (mm)	2.26 ± 0.34^{a}	2.61 ± 0.33^{a}	3.48 ± 0.34^{ab}	4.38 ± 0.36^{b}
WBSF (kg)	5.18 ± 0.54^{a}	4.24 ± 0.54^{a}	5.04 ± 0.51^{a}	5.28 ± 0.51^{a}

^{abc} Means with different literal within row are different (P<0.05.)

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VALIDATION OF ACID INSOLUBLE ASH (AIA) AS AN INTERNAL BIOMARKER FOR DIGESTIBILITY STUDIES IN HARBOR SEALS (*PHOCA VITULINA*).

K.S. Yamamoto*[1], J.R. Carpenter[1], S. Atkinson[2], L. Polasek[2], and H. Zaleski[1]
[1]CTAHR, University of Hawai`i at Manoa, Honolulu, HI, and [2]Alaska SeaLife Center, Seward, AK

ABSTRACT: The purpose of this study was to validate the internal biomarker, acid insoluble ash (AIA), as a replacement for the external marker chromic oxide (Cr2O3) for determining dry matter, protein, lipid and energy digestibility in harbor seals. The AIA assay was assessed using prey and fecal samples from captive harbor seals of varying ages, housed in Alaska, consuming either a single prey (high- or low-fat herring), or mixed prey (herring, squid, pollock and capelin) diets. Prey and fecal samples were analyzed using AIA and Cr2O3 as dietary markers in three separate trials. Digestibility values obtained using AIA as a marker were significantly correlated with results obtained using Cr2O3 when assessing lipid ($r=0.95$, $p<0.0001$), dry matter ($r=0.57$, $p=0.03$) and gross energy ($r=0.83$, $p=0.0002$) in seals consuming a high fat diet, and when assessing protein ($r=0.67$, $p<0.001$), lipid ($r=0.86$, $p<0.0001$) and gross energy ($r=0.62$, $p=0.003$) in seals on a mixed diet. Although mean digestibility for each nutrient across seals did not differ significantly between using AIA and Cr2O3, the protein, lipid, dry matter and gross energy digestibility values obtained with the two methods were not significantly correlated when seals were consuming low fat herring diets. Digestibility of protein for seals fed a high fat diet, and dry matter for seals fed a mixed diet were not correlated ($P>0.05$). Results from these studies suggest that AIA can be used as a reliable marker for determining nutrient digestibility in harbor seals. Although not all nutrient digestibility calculations using the AIA and Cr2O3 methods were significantly correlated for each trial the means across animals within trial did not differ ($P>0.05$) with method used. Compared to the Cr2O3 method, the use of AIA as a marker saves on labor, cost and time, and external markers or unnatural materials are not required in their diets. These results would suggest that there would also be direct application in the determination of digestibility for other marine mammal species as well.

Key Words: Acid insoluble ash (AIA), nutrient digestibility, harbor seals

Introduction

Harbor seals (*Phoca vtiulina)* are widespread in the Atlantic and North Pacific oceans. Over the past 30 years harbor seal numbers have declined in Alaskan waters. Factors causing declines in pinniped populations are unknown. One hypothesis suggests that pinnipeds are experiencing nutritional stress as a result of changes in the quality and availability of prey items (Alverson, 1992; Calkins *et al*,. 1998). In order to assess how changes in prey availability impact marine mammal populations, the nutritional composition of prey items and the way in which marine mammals digest and utilize prey must first be understood.

Digestibility studies have traditionally been performed through the use of either total fecal collection or the use of an indirect dietary indicator or marker method. A marker is a reference compound that may be used to assess physical and chemical aspects of digestion (Owen and Hanson 1992). Markers can be used in digestibility studies to assess how food and nutrients are utilized by a particular animal (Kotb and Luckey, 1972). An ideal marker should be inert, unaltered during passage through the alimentary tract and non-toxic. Markers can be classified as either external or internal (natural) markers (Kotb and Luckey, 1972).

The most commonly used external fecal marker in digestibility studies is chromic oxide (Cr_2O_3). Although Cr_2O_3 has been validated on numerous species (Fernandez *et al.,* 1999; Hill *et al.,* 1996; Corbett *et al.,* 1960; Kotb and Luckey, 1972; Hardison *et al.,* 1955; Kiesling *et al.,* 1969), use of this marker can be problematic (Kotb and Luckey, 1972). The reliability of Cr_2O_3 as a marker is highly dependent upon uniform mixing in the feed. In most marine mammal rations it is difficult to ensure proper mixing of Cr_2O_3 in the diet. Many marine mammals consume whole fish, therefore administration of Cr_2O_3 is often through a capsule placed in the gills of a fish (Goodman-Lowe *et al.,* 1997; Bleakney *et al.,* 2005). The most common internal marker used in digestibility studies is acid insoluble ash (AIA) (Sales and Janssens 2003). AIA is composed of indigestible mineral components and is naturally found in most feed and prey items. Validation of AIA as a reliable marker eliminates the need to confine captive animals.

The objective of this study was to validate the internal biomarker, acid insoluble ash (AIA), as a replacement for the external marker Cr_2O_3 for digestibility (DIG) studies in harbor seals (*Phoca vitulina)* to determine dry matter, protein, lipid and energy digestibility of commonly consumed prey items.

Materials and Methods

This experiment used prey and fecal material from two different studies, from captive harbor seals (*Phoca vitulina*) housed at Alaska SeaLife Center (ASLC) in Seward, AK. Study 1, conducted by Kathryn Stanberry from January to March 2003, focused on DIG of high-fat and low-fat herring diets in harbor seals (Stanberry,

2003). Study 2 included trials in January and August of 2005, January and August of 2006 and January 2007. Harbor seals used in Study 2 consumed a mixed diet consisting of high- or low-fat herring, plus squid, pollock and capelin.

Animals and Feeding

Study #1

This study was performed under a scientific research and enhancement permit from the National Marine Fisheries Service (Permit #881-1443-05) and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Hawaii (Protocol #03-002) and the ASLC (Protocol #00-007-03). The Atlantic and Pacific harbor seals (3 males and 2 female) used for this study resided at the ASLC from 1998 and throughout the duration of the study which was conducted from January - March 2003. The initial body weights of the seals ranged from 53 to 75 kg, and the ages of the seals ranged from 7-20 years old.

During the first 4 weeks of the study the seals were fed Pacific high-fat herring (Table 1), then switched to low-fat herring (Atlantic) for the last 4 weeks. The lot for each species of fish was consistent during the study. Proximate analysis (Table 1) showed a 10% difference in crude fat content between the Pacific and Atlantic herring (Stanberry, 2003). In week 4 of both feeding regimes, fecal samples were collected during a 72-hr DIG trial and nutrient DIG was determined daily (n=3) for each seal.

Study #2

This study was performed under a scientific research and enhancement permit from the National Marine Fisheries Service (Permit #881-1710-06) and was approved by the Institutional Animal Care and Use Committees (IACUC) of the University of Hawaii (Protocol #05-004) and the Alaska SeaLife Center (Protocol #03-009). The juvenile Pacific harbor seals used for this digestibility study were housed at the ASLC throughout the duration of the study (January 2005-January 2007). All seals were females; ranges in body weights were 22-44kg, and ages 1 to 2.5 years.

Diets were composed of 30% high- or low-fat herring (*Clupea harengus),* 10% squid (*Loligo spp),* 30% capelin (*Mallotus villotus),* and 30% pollock (*Theragra chalocogramma*). Sub-samples of prey along with seal fecal samples were collected during the 72-hr DIG trial and kept frozen at -20°C until transferred to Hawaii where they were kept at -65°C until analysis. Daily records of intake (kg) were kept to monitor the amount of food each seal consumed. For both studies, the amount of fish fed to each individual seal was a fixed amount based on the average amount being consumed for the 5-7 day period prior to each study.

Proximate Analysis

Standard methods from the 1990 Association of Official Analytical chemists (AOAC) for meat (Ellis, 1984) were used to determine the amount of moisture, dry matter, ash, lipid and crude protein in both the prey items and fecal samples from the DIG trials. Triplicate analyses were run for each nutrient unless there was not enough sample available. In those cases, duplicate analyses were performed. We used 5% variation as acceptable between replications. Prey items were randomly sampled daily from the seals diets for each trial and frozen. Prior to proximate analysis the prey items were thawed and homogenized into a slurry. After homogenization, the samples from both studies were thoroughly mixed, sub-sampled and put into sterile Blue Max™ MPS 50 ml modified polystyrene conical centrifuge tubes and refrozen at –80°C until analyses were performed.

Dry matter and moisture for the prey items were determined by using 3-5g of wet sample (thawed and homogenized) dried in ashing 40 ml ceramic crucibles placed in a 100ºC drying oven for 24 hrs. Dry fecal and prey samples were placed in an electric muffle furnace at 650°C for 8 hrs to determine ash content. Crude protein for the prey items and fecal samples was analyzed using the traditional or modified Kjeldahl nitrogen analysis. Fresh prey samples and dry fecal samples were digested in sulfuric acid using a Tecatur 1015 block digester programmed with a 4.5 hr ramp up and digestion time. Samples were then distilled and titrated using normal Kjeldahl units and nitrogen determination. Percent nitrogen determined by Kjeldahl analysis was multiplied by the factor 6.25 (or 100/16) to determine crude protein content. Lipid (crude fat) for most prey and fecal samples was determined using the Goldfisch ether extract method. Carbohydrate (CHO) of the prey and fecal samples was calculated using the following equation: %CHO (dry matter basis) = %100 – (% Ash + % Crude Protein + % Lipid). Gross energy was determined from bomb calorimetry using a PARR adiabatic calorimeter.

Administration of Chromic Oxide, Fecal Collection, Analysis of Chromic Oxide and Acid Insoluble Ash (AIA), and Digestibility Calculations

Studies 1 and 2 both used Cr_2O (Fisher Scientific, Fairlawn, N.J.) as an indigestible fecal marker. Chromic oxide capsules were distributed throughout each feeding period in gel capsules placed in the opercular cavity of the fish. In order to equilibrate in the gastrointestinal tract, Cr_2O_3 was given to seals two to four days before the start of each 72-hr collection trial and distributed in two daily feeds during dry holding. For both studies, each seal was given Cr_2O_3 at the rate of 0.3% of the DM consumed.

After animals were placed in raised holding cages to start the 72hr fecal collections, staff monitored the seals through a live feed camera, or made very frequent visual checks for the duration of the collection period. Seals were sprayed with a hose periodically to ensure proper thermoregulation. Each defecation was collected, weighed and placed in Ziploc bags. Weight of the sample and time of defecation were recorded and samples immediately placed in a –20ºC freezer. Fecal samples were later placed in a large cooler with ice packs and transported from Alaska to Hawaii for analysis.

At time of analysis, the weight of the frozen fecal samples was recorded and the samples transferred into glass pie pans and placed into a 50ºC dry oven for 72 hrs.

Hill and Anderson's (1958) nitric acid and perchloric acid digestion procedures were used to analyze the Cr_2O_3 content for each fecal sample, which had been dried and ground through a Wiley mill 1mm stainless steel screen. Fecal samples were analyzed in duplicate and 0.50 to 0.75g of ground fecal sample was used for each analysis. A spectrophotometer (BioRad Smart Spec 3000; BioRad Laboratories, Hercules, CA) set at 444 nm was used to read the results. A standard regression equation was developed using, 2.5, 5, 10, 15, 20, and 25 mg of Cr_2O_3.

Acid insoluble ash (AIA) was determined for both the prey and fecal samples using a modification of the Van Keulen and Young (1977) 2N HCl. Preliminary assays were run with different samples of prey and feces at various drying and ashing temperatures to determine the best method for this study. Eight grams of dried and ground feed or feces was placed in a 40 ml crucible and dried in a forced air oven at 100ºC. After 24 hrs, the sample was cooled in a desiccator, reweighed and ashed for 8 hrs at 650ºC. The ashed sample was weighed and placed in a 600 ml Berzelius beaker and mixed with 100 ml of 2N HCl. The mixture was boiled for 5 minutes on a hotplate with a condenser attached to ensure that HCl was not lost. The hot hydrolysate was filtered using Whatman No. 41 ashless filter paper and washed with hot distilled water to remove excess acid. The ash and filter paper were placed back into the 40 ml crucible and both were ashed for 8 hrs at 450ºC. The crucible was cooled in a desiccator and weighed. The ash from the crucible was removed and the crucible was immediately re-weighed. All fecal AIA analyses were corrected for the chromic oxide content.

Data Analysis

Data were analyzed using Microsoft Excel and Statistical Analysis System (SAS) Software (SAS Institute Inc., Cary, North Carolina). Excel was used to calculate the percent Cr_2O_3 and AIA in the prey and fecal samples. SAS was used to run correlations to compare DIG results obtained with the AIA assay method to DIG results that were determined by using the external indicator Cr_2O_3. Spearman and Pearson correlations were used to determine the influence of outliers on correlations.

Results and Discussion

Nutrient composition of the various prey items consumed by seals in both studies is listed in Table 1. Percent DM for both high-fat and low-fat herring in Study 1 were similar to dry matter values in Study 2. Percent AIA was similar for low-fat herring in Studies 1 and 2 but varied for high-fat herring between the two studies.

Study #1: Harbor seals on high- and low-fat herring diets

Digestibility (DIG) of the various nutrients was relatively high (>90.0%) when Cr_2O_3 and AIA were used as markers (Tables 2 and 3), and values obtained with Cr_2O_3were similar to results obtained using AIA as an internal marker. Although the average DIG values obtained using the Cr_2O_3 and AIA method were similar, the values were not always significantly correlated with one another. For the high-fat herring diet lipid DIG ($r=.95$, $p < .0001$) values, dry matter DIG values ($r=0.57$, $p =0.03$) and digestible energy DIG values ($r=.83$, $p = .0002$) were significantly correlated to AIA (Table 2). However, DIG of protein using the two different methods were not significantly correlated ($r=.38$, $p=.19$). For the low-fat herring diet (Table 3), the DIG protein, lipid, DM, and energy values obtained using the AIA marker were not significantly correlated with values obtained using Cr_2O_3. Spearman and Pearson correlations indicated that outliers did not significantly alter correlations.

Study #2: Harbor seals on a mixed prey diet

Like study 1, the protein, lipid and digestible energy values were relatively high for the harbor seals on a mixed prey diet (Table 4). With the exception of dry matter, average nutrient DIG obtained when AIA was used as a marker were similar to results obtained with Cr_2O_3. DIG values obtained using the AIA method were significantly correlated to those obtained using the Cr_2O_3 method, with the exception of DM. The highest correlation between the two different methods was lipid DIG ($r = 0.86$, $p < .0001$), followed by protein DIG ($r=.68$, $p = .0009$) and digestible energy ($r = .62$, $p = .003$). Dry matter DIG was not significantly correlated ($p=0.21$). Like Study 1, Spearman and Pearson correlations indicated that outliers did not significantly alter these correlations. The various nutrient DIG determined in this study were similar to those determined in other pinniped research on herring diets (Ashwell-Erickson & Elsner, 1981; Rosen, 1996. Overall DM DIG was significant but low ($r=0.57$) when compared with protein, lipid and energy for high fat herring in Study 1. In Study 1, feces obtained from seals on the low-fat herring diet contained 13.5% more ash than feces from seals on the high fat herring diet (Stanberry, 2003).

The overall mean values obtained from Studies 1 and 2 suggest that AIA can be used as a reliable marker for determining nutrient digestibility in harbor seals that consume mixed prey or single prey (high-fat herring) diets for protein, lipid and energy. The use of a natural internal biomarker such as AIA has been reported to provide many benefits over the use of Cr_2O_3 and has been used successfully in multiple species (Kotb and Luckey, 1972, Sales and Janssens, 2003). This study marks the first time that the AIA assay has been performed and validated on a marine mammal. AIA saves time and reduces the labor involved for collection of fecal and prey samples, and analysis of these samples in the laboratory. AIA studies only require representative samples of feed and feces rather than quantitative measures and total fecal collections, saving time and cost for analysis. When using Cr_2O_3 as an indicator, hazardous chemicals such as concentrated nitric acid and perchloric acid are used during sample analysis. A sulfuric acid digestion mixture is also used to analyze samples. The lower normality of the 2N HCl acid makes the AIA method safer than the Cr_2O_3 method when analyzing samples in the laboratory (El Hag and El Hag, 1983). Analysis using the Cr_2O_3 method can be difficult, time consuming and tedious (Kotb and Luckey, 1972). AIA lowers the impact on animals because AIA is a natural marker. Natural markers

are more accurate than external markers that are added to diets (Kotb and Luckey, 1972) and are also less likely to interfere with or affect the digestive physiology of the animal being tested (Talbot, 1985). Chromic oxide and other external markers must be added to the feed or consumed in boluses; therefore, mixing and distribution of the marker within the gastrointestinal tract may not be uniform. Since AIA is naturally found in all feedstuffs, and prey items, uniform distribution is not a concern.

Conclusions and Implications

DIG results from these trials suggest that AIA can be used as a reliable marker for determining nutrient digestibility in harbor seals that consume single prey (high-fat herring) and mixed prey diets. Overall, this study showed that AIA is a valuable marker that should be considered for use in digestibility studies instead of the traditional Cr_2O_3 marker. AIA has been used successfully in multiple species and has been reported to provide many benefits over the total fecal collection method and external markers such as Cr_2O_3. Compared with the chromic oxide method, the use of AIA as a marker in digestibility studies saves on time, labor and money. The AIA assay also minimizes the use of strong and dangerous acids during analysis. Digestibility studies using AIA as a marker can easily be performed on captive and wild marine mammals if the components of the diets are known. A sub-sample of each prey item consumed, and sub-samples of the feces could provide an approximation of marine mammal DIG. Knowledge of DIG would help to assess the nutrient requirements of marine mammals and the effects of changes in prey availability on wild marine mammal populations.

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Table 1. Mean nutrient composition (± SEM) of prey items fed (DMB), for both digestibility studies.

Study	Nutrient	% Dry Matter	EE (%)	Ash (%)	Protein (%)	Gross Energy (kcal/g)	AIA (%)
#1	High-Fat Herring (n=2)	32.6 ±1.3	43.9 ± 3.2	7.0 ± 0.4	46.7± 1.0	6.91 ± 0.1	0.24 ± 0.01
	Low-Fat Herring (n=2)	22.2 ±0.5	16.0 ± 2.6	8.6 ± 0.3	73.6± 0.6	5.70 ± 0.2	0.26 ± 0.1
#2	High-Fat Herring (n=37)	29.7 ±0.2	38.6 ± 0.7	8.0 ± 0.1	61.5 ± 0.9	6.19 ± 0.1	0.15 ± 0.0
	Low-Fat Herring (n=31)	25.5 ±0.3	23.6 ± 1.2	10.7 ± 0.2	71.9 ± 1.1	5.63 ± 0.0	0.23 ± 0.0
	Capelin (n=33)	19.5 ±0.2	14.8 ± 0.5	11.5 ± 0.2	85.3 ± 0.8	5.30 ± 0.1	0.28 ± 0.0
	Pollock (n=45)	22.9 ±0.3	20.5 ± 1.4	12.0 ± 0.3	76.0 ± 1.5	5.50 ± 0.1	0.31 ± 0.1
	Squid (n=24)	19.9 ±0.2	7.4 ± 0.8	8.3 ± 0.3	86.0 ± 0.8	5.16 ± 0.1	0.37 ± 0.1

Table 2. Comparison of AIA (internal) vs Cr_2O_3 (external) marker for determining protein, lipid, dry matter and gross energy digestibility in mature harbor seals fed only high-fat herring (all data are mean percent ± SD, DMB).

Seal	Protein		Lipid		Dry Matter		Gross Energy	
n = 3 / seal	AIA	Cr_2O_3	AIA	Cr_2O_3	AIA	Cr_2O_3	AIA	Cr_2O_3
Tina	96.8 ± 0.8	94.9 ± 0.9	96.8 ± 0.8	94.9 ± 0.9	96.0 ± 1.0	93.5 ± 1.3	97.9 ± 0.7	96.6 ± 0.9
Cecil	95.5 ± 0.8	95.9 ± 0.5	99.3 ± 0.1	99.4 ± 0.1	90.4 ± 1.3	91.2 ± 0.3	94.4 ± 2.8	94.9 ± 4.3
Snapper	94.7 ± 1.2	95.1 ± 1.0	99.0 ± 0.3	99.1 ± 0.2	90.4 ± 1.3	91.2 ± 0.3	94.4 ± 4.8	94.9± 4.3
Pender	94.5 ± 2.0	93.8 ± 0.9	96.9 ± 2.0	95.8 ± 3.9	90.2 ± 2.2	91.0 ± 0.3	96.5 ± 0.9	96.8 ± 0.4
Sydney	95.8 ± 0.7	97.3 ± 1.8	98.8 ± 0.3	99.1 ± 0.4	87.4± 1.2	90.8 ± 1.0	96.0 ± 0.5	97.1 ± 0.2
OVERALL Mean	95.4 ± 1.4	95.2 ± 1.4	98.6 ± 1.3	97.4 ± 2.1	90.7 ± 3.5	90.6 ± 3.0	96.1 ± 2.4	95.9± 2.3
r value	0.38		0.95		0.57		0.83	
p value	0.19		<.0001		0.0339		0.0002	

Table 3. Comparison of AIA (internal) vs Cr_2O_3 (external) marker for determining protein, lipid, dry matter and gross energy digestibility in mature harbor seals fed only low-fat herring (all data are mean percent ± SD, DMB).

Seal	Protein		Lipid		Dry Matter		Gross Energy	
n = 3 / seal	AIA	Cr_2O_3	AIA	Cr_2O_3	AIA	Cr_2O_3	AIA	Cr_2O_3
Tina	96.3 ± 0.4	93.9 ± 3.2	98.5 ± 0.6	97.2 ± 2.6	89.1 ± 2.7	80.8± 13.5	96.2 ± 0.7	93.5 ± 3.8
Cecil	94.4 ± 2.3	92.0 ± 2.0	97.6± 1.2	96.6 ± 1.0	75.9 ± 6.9	65.2± 7.3	93.5 ± 2.7	90.8 ± 2.2
Snapper	92.6 ± 1.6	94.6 ± 2.2	96.5 ± 0.3	97.2 ± 1.7	67.1 ± 8.2	76.5 ± 8.8	91.3 ± 1.7	93.6 ± 2.9
Pender	95.8 ± 0.8	95.7 ± 0.2	97.9 ± 0.9	98.0± 0.6	79.2 ± 4.1	79.0 ± 1.6	94.6 ± 0.9	94.6 ± 0.2
Sydney	91.3± 1.8	95.5 ± 1.0	96.0 ± 2.0	97.9 ± 1.0	56.3 ± 8.0	77.1 ± 4.6	90.4± 2.3	95.0± 1.2
OVERALL Mean	94.1 ± 2.3	94.3 ± 2.2	97.3 ± 1.4	97.4 ± 1.4	73.5 ±12.7	75.7 ± 9.0	93.2 ± 2.7	93.5 ± 2.5
r value	-0.1995		0.2073		0.0217		-0.1599	
p value	0.4759		0.4584		0.9387		0.5692	

Table 4. Comparison of AIA (internal) vs Cr_2O_3 (external) marker for determining protein, lipid, dry matter and gross energy digestibility in juvenile harbor seals fed mixed prey diets (all data are mean percent ± SD, DMB).

Seal	Protein		Lipid		Dry Matter		Gross Energy	
Seal (Diet); (n)	AIA	Cr_2O_3	AIA	Cr_2O_3	AIA	Cr_2O_3	AIA	Cr_2O_3
Qilak (HF); (4)	95.7 ± 0.8	97.0 ± 0.3	99.1 ± 0.2	99.4 ± 0.2	80.0 ± 3.4	86.1± 1.6	95.2 ± 0.8	96.7 ± 0.3
Anya (HF); (2)	95.9 ± 1.7	97.5 ± 0.5	99.3 ± 0.2	99.6 ± 0.0	82.8 ± 6.4	90.3± 1.9	95.6± 1.7	97.4 ± 0.5
Atuun (HF); (2)	96.2 ± 2.4	97.2 ± 0.8	99.8 ± 0.1	99.8 ± 0.0	87.4 ± 5.4	89.5 ± 0.3	96.3 ± 2.1	97.1 ± 0.5
Siku (HF); (1)	95.7 *	97.4 *	99.1*	99.5 *	75.7*	85.3*	94.6*	96.8*
Mean (HF)	95.8 ± 1.8	97.2 ± 0.6	99.3 ± 0.4	99.6 ± 0.3	82.0 ± 7.1	87.7 ± 3.0	95.5 ± 1.8	97.0 ± 0.6
Miki (LF); (4)	94.8 ± 1.2	96.5 ± 0.2	98.2 ± 0.7	98.9 ± 0.4	78.5 ± 4.6	85.5 ± 0.5	93.6 ± 1.4	95.7 ± 0.2
Susitna(LF); (5)	95.1± 1.6	96.9 ± 0.2	98.5 ± 0.5	99.0 ± 0.1	79.7 ± 5.2	85.9 ± 0.9	94.2± 1.8	96.1 ± 0.1
Shila (LF); (2)	97.1± 0.2	97.8 ± 0.1	98.7 ± 0.5	99.0 ± 0.4	82.5 ± 3.9	86.6 ± 2.6	96.2± 0.5	97.1± 0.3
Tikanni(LF);(1)	94.2*	96.4*	99.8*	99.9*	74.0*	83.9*	90.6*	94.2*
Mean (LF)	95.3 ± 2.6	96.9 ± 0.5	98.5 ± 1.1	99.0 ± 0.6	79.3 ± 8.9	85.7 ± 1.8	94.0 ± 3.1	96.0 ± 0.8

HF = high-fat diet; LF = low-fat diet

* SD n/a; only one sample.

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INFLUENCE OF DESCENDING DIETARY-PROTEIN LEVELS ON PERFORMANCE OF FATTENING JAPANESE QUAIL: I. RESPONSE DURING THE HOT SEASON

J.J. Lomeli, L.R. Flores and R. Barajas

Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Sinaloa, México.
rubar@uas.uasnet.mx

ABSTRACT: This experiment was conducted with the objective of determine the influence of descending dietary-protein levels on performance of fattening Japanese quail during the hot season. Six hundred one day old Japanese quail (9.5 ± .07 g) were used in a 28-days fattening-experiment. Quails in groups of fifty were placed in twelve metallic-wire cages and were randomly assigned to receive one of three treatments: 1) 24% Corn-soybean meal-based diet (ME = 2,950 Kcal/kg) during 28-days fattening period (CTRL); 2) 24% CP-Diet during first 21 days, and then changed to a 21% CP-Diet (ME = 3,040 Kcal/kg) remainder 7 days (21D); and 3) 24% CP-Diet during first 14 days, and then changed to a 21% CP-diet remainder 14 days (14D). Ending weight (185.4 ± 1.76 g), and total weight gain (175.9 ± 1.75 g) were similar across treatments (P > .60). Feed intake (452.2 ± 4.37 g by quail) was not affected (P = .17) by treatments. Feed efficiency (0.387 ± .01) was not influenced by dietary CP level (P = .40). CP intake was decreasing (P < .01) as time in 24% CP-diet was reduced with values of 109.4, 104.4 and 97.7 g by quail, for treatments CTRL, 21 and 14, respectively. During third week quails fed 21% CP-Diet (14D) exhibit higher (P < .01) CP-efficiency (gain, g/CP intake, g) than animals receiving 24% CP-Diet (CRTL and 21D), with values of 1.91 vs. 1.67 g of gain for each g of CP intake, for 21% vs. 24% CP-Diet, respectively. During last week, animals receiving 21% CP-Diet (14D and 21D) had higher CP-efficiency (P < .01) than animals fed 24% CP diet, with mean values of 1.15 vs. 0.98, for 21% vs. 24% CP-Diet, respectively. Complete experiment crude protein efficiency was increased (P < .01) in 8% (1.79 vs. 1.65) fed 24% CP-diet during first 14 days only and then changed to 21% CP-Diet compared with remainder feeding schedules (CTRL and 21D). Carcass Wt. was not affected (P = .31). It is concluded that feeding 24% CP-diet during 14 days and changed to 21% CP-Diet has not detrimental effects on performance of fattening Japanese quail during hot season and save protein expenses.

Key words: Japanese quail, Growth-performance, Protein.

Introduction

Usually it is accepted that birds requires less protein as its age is increased (Heuser, 1941; Boon et al., 2001). Agree with this, is recommended a decrement in dietary crude protein level as animals advances in age and productive phase, and the principle is applied to Poultry, Ducks, Turkeys, Pheasants, and include for Bob-white quail (NRC, 1994). However, for Japanese quail the actual recommendation is the same 24% CP-level both during starting and growing period (NRC, 1994). Adequacy of a constant 24% CP-level is supported by several experiments performed with unselected lines of Japanese quail (Weber and Reid, 1967; Lepore and Marks, 1968; Vhora and Roudybush. 1971). Conversely, experiment conducted with Japanese quail selected for heavy weight, do not found differences on body weight at five week age in animals fed 24% or 21% CP-diets (Marks, 1993). Using a descending dietary-CP schedule (Moritsu et al., 1997), Japanese quail fed with a 29% CP-diet during first two weeks and then changed to an 18% CP-diet observed that quails selected for high body weight arrive at 195 g of body weight in four weeks, while low performance lines obtain only 42 g of body weight in the same time. Barajas et al. (2004), testing two descending CP-diet schedules against to constant 24% CP-diet feeding high weight-selected Japanese quail, alternative treatments were 28% or 24% CP-diet during first week, 24% CP-diet during second week and 21% CP-diet during third and fourth weeks in both treatments, ending weight (198 g) and feed efficiency were similar across treatments, but during third week weight gain was lower in quails fed 21% CP-diet compared with animals eating 24% CP-diet. Literature information exposed that CP requirements during ending fattening period is lower than at starting, however the best time to decrease dietary CP-level is not completely clear. This research was conducted with the objective of determine the influence of descending dietary-protein levels on performance of fattening Japanese quail during the hot season.

Material and Methods

Location

The experiment was performed from September 14 to October 10, 2006 in the facilities of the Facultad de Medicina Veterinaria y Zootecnia, of the Universidad Autonoma de Sinaloa, in Culiacan, Sinaloa, situated in Northwest Mexico (24º 46' N. and 107º 21' W. ; 80 m

o.m.s.l.; mean temperature 25 ºC, and 645 mm annual rainfall.

During 28 days experiment mean temperature inside of barn was 29.67 ± 1.53 ºC, ranking from 36.1 to 21.5 ºC, and relative humidity was 69.65 ± 2.79%, ranking from 90 to 48%.

Animals

Animals used in the experiment were managed according to the recommended guidelines in *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1988). Six hundred one day old unsexed Japanese quail (9.5 ± .07 g) of a high body weight selected-line were used in a 28-days fattening-experiment. Quails were divided in groups of fifty, weighed and placed in twelve metallic-wire cages (0.9 x 0.9 x 0.5 m). During first seven days a metallic network was provided as bed for small quails. Cages were fitted with metallic feed bunker and a 3.78 L plastic-drinker that was refilled twice a day to guarantee permanently access to clean and fresh water. First five days ciprofloxacin (75 mg/L) was added as prophylactic antimicrobial agent. During first four days food was offered in a 0.19 x 0.28 m type-Pan feeder, and then changed to metallic feed bunk. Electric light bulb were used to provide adequate temperature inside of cages, 40 ºC first three days, 35 ºC remainder first week, and reduced weekly 5 ºC rest of experiment. Light was turn on 24 hours of day.

Design and Treatments

Agree with a completely randomized design (Hicks, 1973) were assigned to receive one of three feeding schedules in that consists the treatments: 1) 24% Corn-soybean meal-based diet (ME = 2,950 Kcal/kg) during 28-days fattening period (CTRL); 2) 24% CP-Diet during first 21 days, and then changed to a 21% CP-Diet (ME = 3,040 Kcal/kg) remainder 7 days (21D); and 3) 24% CP-Diet during first 14 days, and then changed to a 21% CP-diet remainder 14 days (14D). Composition of the diet is presented in Table 1. Food was weighed and manually delivery twice a day (0800 and 1600), and refusals were removed and weighed daily (0800). Animals were weighed weekly. Mortality was recorded daily and death animals were not replaced.

Upon complete fattening period, three males and three females for cage were randomly selected (six quails for cage; 24 by treatment) to be sacrificed by decapitation, after that, feathers, skin, foots, and wings were removed and carcass weight was obtained.

Statistical

Experiment was analyzed by ANOVA procedure as a completely randomized design (Hicks, 1973), for performance variables each cage was consider as the experimental unit, while for carcass measurements each carcass constituted a repetition, an alpha level of 0.05 was fitted to accept statistical difference, if existed means were compared using Tukey test (Hicks, 1973). Direct comparison between CP-diets level for third and fourth week data were performed using orthogonal contrast process. Linear or quadratic trend was tested by polynomial contrast procedure. All statistical calculation was conducted using version 8 of software Statistix™ (2003).

Table 1. Composition of the diets used in the experiment

Ingredients	Treatments Dietary CP content, %	
	24	21
Soybean meal	35.0	26.0
Ground corn	55.0	63.7
Vegetable oil	1.7	1.5
Fish meal	7.0	7.0
Limestone	0.95	1.16
Salt	0.3	0.3
Mineral and Vitamin premix	0.25	0.25
L-Lysine	-	0.055
DL- Methionine	-	0.035
Total	100 %	100 %
Calculated Analyses [1]		
Crude protein, %	24.00	21.00
ME, Mcal/kg	2.95	3.04
Lys, %	1.49	1.30
Met + Cis, %	0.83	0.77
Ca, %	0.80	0.84
Available P, %	0.35	0.34

[1] Calculated from Publisher values (NRC, 1994).

Results and Discussion

The effect of different feeding schedules on performance and carcass weight of fattening Japanese quail is shown in Table 2. During complete 28 days experiment, ending body weight (185.4 ± 1.76 g) and body weight gain (175.9 ± 1.75 g) were similar across feeding schedule (P > .60). Feed intake (452.2 ± 4.37 g) and Feed efficiency (0.387± .01 g of gain/g of food) were not affected by treatments (P > .15). Carcass weight (120.2 ± 1.88 g) and hot carcass dressing (62.08 ± 0.36%) were not influenced by dietary-CP feeding schedules (P > .30). This results confirms the hypothesis that Japanese quail do not requires necessarily consume diet with 24% of CP during complete four weeks fattening period, and is in concordance with reported by Barajas et al. (2004).

During third and fourth weeks quails fed 21% CP-diet ate 13.7% and 15.3% less protein (P < .01), respectively than animals consuming 24% CP diet. Over complete experiment, total intake CP decreased linearly as the time in 24% CP-diet was reduced (P < .01). Japanese quail fed 24% CP-diet only during their first 14 days of live consumed 11.7 g of CP less (10.7%) than quails fed 24% CP-diet during 28 days (P < .01), and 6.4% fewer

than animals fed 24% CP-diet during 21 days. These results coupled with lack effect on feed intake (P = .17), outcomes in an enhancement in CP efficiency, that was improved (P < .01) in 14.7% and 17% for 21% CP-diet in relationship with quails ate 24% CP-diet during third and fourth weeks, respectively.

Over complete 28 days fattening experiment, the CP efficiency (g of body gain by each g of CP intake) was 10.5% higher (P < .01) in Japanese quail that consumed 14 days 24% CP-diet and remainder 14 days ate a 21% CP-diet (1.79 vs. 1.65 g/g), compared with animals fed 24% CP-diet during more time (21 or 28 days). This result is in concordance with values of protein efficiency equivalents of 1.82 and 1.54 observed by Marks (1971) when fed Japanese quails with diets containing 21 or 24% of CP, respectively.

Boon et al. (2001) explain that CP is required in young Japanese quails to construct new tissues specially muscle and bone that support mostly of growth impetus. Data from this experiment shown that quails increased its weight 19 times during fattening period, but the most explosive growth rate is observed during first and second weeks, when quails increased 4.1 and 2.3 times its body mass, respectively, then growth rate is decreased to proportional weekly increments of 1.6 and finally 1.3 times during weeks three and four, respectively. Then agree with Boon et al. (2001), if growth rate is diminished needs of protein for muscle building is decreased too.

Implications

Results of this research suggest that fattening Japanese quail after fourteen days of life decreased its growth impetus and concomitant protein requirements, so that diets containing less than 24% of crude protein could be offered without detrimental effects on growth performance. Proportioning diets containing near of 21% of crude protein during days 15 to 28 to fattening Japanese quail supply enough protein to maintain growth performance, and helps to save close of 10% in protein expenses.

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Table 2. Influence of descending dietary-protein level on performance of growing Japanese quail during hot season (September-October, 2006).

Item	Treatments [1] Length in 24% CP diet, days			SEM [2]	*P*-value
	14	21	28		
Cages, replicates, n [3]	4	4	4		
Days in trial	28	28	28		
Weight, g					
Day 1	9.5	9.6	9.5	.07	.22
Day 7	39.2	39.4	39.3	.29	.88
Day 14	89.2	88.9	91.0	.74	.15
Day 21	143.3	144.0	145.5	.87	.25
Day 28	184.3	185.1	186.8	1.76	.61
Weight gain, g					
Week 1	29.7	29.8	29.8	.26	.99
Week 2	51.0	49.5	51.8	.62	.08
Week 3	54.1	55.1	54.5	.58	.50
Week 4	41.0	41.1	41.3	1.11	.98
All 4 weeks	174.8	175.5	177.3	1.75	.60
Feed Intake, g					
Week 1	42.0	43.5	42.0	.71	.27
Week 2	99.8	103.2	102.4	1.56	.31
Week 3	135.1	138.1	136.1	1.51	.41
Week 4	168.0	171.5	175.2	2.43	.17
All 4 weeks	445.0	456.3	455.3	4.37	.17
Feed efficiency, gain g/feed intake g					
Week 1	.71	.68	.71	.01	.35
Week 2	.50	.48	.51	.01	.17
Week 3	.40	.40	.40	.01	.98
Week 4	.24	.24	.24	.01	.65
All 4 weeks	.39	.38	.39	.01	.40
CP Intake, g					
Week 1	10.1	10.5	10.1	.17	.27
Week 2	24.0	24.8	24.6	.37	.31
Week 3 [4, 5]	28.4 b	33.1 a	32.7 a	.33	< .01
Week 4 [4, 5]	35.3 b	36.0 b	42.1 a	.52	.02
All 4 weeks [4, 5]	97.7 c	104.4 b	109.4 a	..98	< .01
CP efficiency, gain g/CP intake g					
Week 1	2.95	2.85	2.95	.05	.35
Week 2	2.09	2.00	2.11	.04	.17
Week 3 [4, 5]	1.91 a	1.66 b	1.67 b	.02	< .01
Week 4 [4, 5]	1.16 a	1.14 a	.98 b	.03	< .01
All 4 weeks [4, 5]	1.79 a	1.68 b	1.62 b	.02	< .01
Mortality, %	3.0	5.5	5.0	1.28	.38
Carcass wt, g [6]	119.6	122.2	118.9	1.88	.31
Carcass dressing, % [6]	61.89	62.10	62.25	.36	.77

[1] Treatments: 1) First 14 days fed 24% CP-diet and remainder 14 days fed 21% CP-diet; 2) First 21 days 24% CP-diet and remainder 7 days fed 21% CP-diet; and 3) 24 % CP-diet during 28 days experiment.

[2] Standard error of the mean.

[3] Mean of 50 quails by cage

[4] Linear trend $P < .01$

[5] CP 24% vs. CP 21%, $P < .01$

[6] Means of 24 quails sample by treatment, constituted by 12 males and 12 females

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INFLUENCE OF DESCENDING DIETARY-PROTEIN LEVELS ON PERFORMANCE OF FATTENING JAPANESE QUAIL: II. RESPONSE DURING THE COOL SEASON

J.J. Lomeli, L.R. Flores and R. Barajas

Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Sinaloa, México.
rubar@uas.uasnet.mx

ABSTRACT: This experiment was conducted with the objective of determine the influence of descending dietary-protein levels on performance of fattening Japanese quail during the cool season. Six hundred one day old Japanese quail (9.5 ± .07 g) were used in a 28-days fattening-experiment. Quails in groups of fifty were placed in twelve metallic-wire cages and were randomly assigned to receive one of three treatments: 1) 24% Corn-soybean meal-based diet (ME = 2,950 Kcal/kg) during 28-days fattening period (CTRL); 2) 24% CP-Diet during first 21 days, and then changed to a 21% CP-Diet (ME = 3,040 Kcal/kg) remainder 7 days (21D); and 3) 24% CP-Diet during first 14 days, and then changed to a 21% CP-diet remainder 14 days (14D). Ending weight (203.8 ± 2.0 g), and total weight gain (193.9 ± 2. g) were similar across treatments (P > .50). Feed intake (515.6 ± 5.83 g by quail) was not affected (P = .29) by treatments. Feed efficiency (0.377 ± .01) was not influenced by dietary CP level (P = .28). CP intake was decreasing (P < .01) as time in 24% CP-diet was reduced with values of 123.6, 119.6 and 111.6 g by quail, for treatments CTRL, 21 and 14, respectively. During third week quails fed 21% CP-Diet (14D) exhibit higher (P < .01) CP-efficiency (gain, g/CP intake, g) than animals receiving 24% CP-Diet (CRTL and 21D), with values of 1.77 vs. 1.51 g of gain for each g of CP intake, for 21% vs. 24% CP-Diet, respectively. During last week, animals receiving 21% CP-Diet (14D and 21D) had higher CP-efficiency (P < .05) than animals fed 24% CP diet, with mean values of 1.10 vs. 0.99, for 21% vs. 24% CP-Diet, respectively. Complete experiment highest crude protein efficiency (P < .01) was for 14D treatment, followed by 21D, and the lowest was for CTRL, with values of 1.73, 1.64 and 1.57 g of gain by g of intake protein, respectively. Carcass Wt. was not affected (P = .25). It is concluded that feeding 24% CP-diet during 14 days and changed to 21% CP-Diet has not detrimental effects on performance of fattening Japanese quail during cool season and save protein expenses.

Key words: Japanese quail, Growth-performance, Protein.

Introduction

It is generally accepted that birds needs less protein as its age is increased (Heuser, 1941; Boon et al., 2001). Agree with this, is recommended a decrement in dietary crude protein level as animals advances in age and productive phase, and the principle is applied to Poultry, Ducks, Turkeys, Pheasants, and include for Bob-white quail (NRC, 1994). However, for Japanese quail the actual recommendation is the same 24% CP-level both during starting and growing period (NRC, 1994). Adequacy of a constant 24% CP-level is supported by several experiments performed with unselected lines of Japanese quail (Weber and Reid, 1967; Lepore and Marks, 1968; Vhora and Roudybush. 1971). Conversely, experiment conducted with Japanese quail selected for heavy weight, do not found differences on body weight at five week age in animals fed 24% or 21% CP-diets (Marks, 1993). Using a descending dietary-CP schedule (Moritsu et al., 1997), Japanese quail fed with a 29% CP-diet during first two weeks and the changed to an 18% CP-diet observed that quails selected for high body weight arrive at 195 g of body weight in four weeks, while low performance lines obtain only 42 g of body weight in the same time. Barajas et al. (2004), testing two descending CP-diet schedules against to constant 24% CP-diet feeding high weight-selected Japanese quail, alternative treatments were 28% or 24% CP-diet during first week, 24% CP-diet during second week and 21% CP-diet during third and fourth weeks in both treatments, ending weight (198 g) and feed efficiency were similar across treatments, but during third week weight gain was lower in quails fed 21% CP-diet compared with animals eating 24% CP-diet. Literature information exposed that CP requirements during ending fattening period is lower than at starting, however the best time to decrease dietary CP-level is not completely clear. This research was conducted with the objective of determine the influence of descending dietary-protein levels on performance of fattening Japanese quail during the cool season.

Material and Methods

Location

The experiment was performed in March, 2007 in the facilities of the Facultad de Medicina Veterinaria y Zootecnia, of the Universidad Autonoma de Sinaloa, in Culiacan, Sinaloa, situated in Northwest Mexico (24º 46' N. and 107º 21' W. ; 80 m o.m.s.l.; mean temperature 25 ºC, and 645 mm annual rainfall.

Animals

Animals used in the experiment were managed according to the recommended guidelines in *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (1988). Six hundred one day old unsexed Japanese quail (9.5 ± .07 g) of a high body weight selected-line were used in a 28-days fattening-experiment. Quails were divided in groups of fifty, weighed and placed in twelve metallic-wire cages (0.9 x 0.9 x 0.5 m). During first seven days a metallic network was provided as bed for small quails. Cages were fitted with metallic feed bunker and a 3.78 L plastic-drinker that was refilled twice a day to guarantee permanently access to clean and fresh water. First five days ciprofloxacin (75 mg/L) was added as prophylactic antimicrobial agent. During first four days food was offered in a 0.19 x 0.28 m type-Pan feeder, and then changed to metallic feed bunk. Electric light bulb were used to provide adequate temperature inside of cages, 40 ºC first three days, 35 ºC remainder first week, and reduced weekly 5 ºC rest of experiment. Light was turn on 24 hours of day.

Design and Treatments

Agree with a completely randomized design (Hicks, 1973) were assigned to receive one of three feeding schedules in that consists the treatments: 1) 24% Corn-soybean meal-based diet (ME = 2,950 Kcal/kg) during 28-days fattening period (CTRL); 2) 24% CP-Diet during first 21 days, and then changed to a 21% CP-Diet (ME = 3,040 Kcal/kg) remainder 7 days (21D); and 3) 24% CP-Diet during first 14 days, and then changed to a 21% CP-diet remainder 14 days (14D). Composition of the diet is presented in Table 1. Food was weighed and manually delivery twice a day (0800 and 1600), and refusals were removed and weighed daily (0800). Animals were weighed weekly. Mortality was recorded daily and death animals were not replaced.

Upon complete fattening period, three males and three females for cage were randomly selected (six quails for cage; 24 by treatment) to be sacrificed by decapitation, after that, feathers, skin, foots, and wings were removed and carcass weight was obtained.

Statistical

Experiment was analyzed by ANOVA procedure as a completely randomized design (Hicks, 1973), for performance variables each cage was consider as the experimental unit, while for carcass measurements each carcass constituted a repetition, an alpha level of 0.05 was fitted to accept statistical difference, if existed means were compared using Tukey test (Hicks, 1973). Direct comparison between CP-diets level for third and fourth week data were performed using orthogonal contrast process. Linear or quadratic trend was tested by polynomial contrast procedure. All statistical calculation was conducted using version 8 of software Statistix™ (2003).

Table 1. Composition of the diets used in the experiment

Ingredients	Treatments Dietary CP content, %	
	24	21
Soybean meal	35.0	26.0
Ground corn	55.0	63.7
Vegetable oil	1.7	1.5
Fish meal	7.0	7.0
Limestone	0.95	1.16
Salt	0.3	0.3
Mineral and Vitamin premix	0.25	0.25
L-Lysine	-	0.055
DL- Methionine	-	0.035
Total	100 %	100 %
Calculated Analyses [1]		
Crude protein, %	24.00	21.00
ME, Mcal/kg	2.95	3.04
Lys, %	1.49	1.30
Met + Cis, %	0.83	0.77
Ca, %	0.80	0.84
Available P, %	0.35	0.34

[1] Calculated from Publisher values (NRC, 1994).

Results and Discussion

The effect of different feeding schedules on performance and carcass weight of fattening Japanese quail is shown in Table 2. During complete 28 days experiment, ending body weight (203.8 ± 2.0 g) and body weight gain (193.87 ± 2.0 g) were similar across feeding schedule ($P > .55$). Feed intake (515.6 ± 5.83 g) and Feed efficiency (0.377± .01 g of gain/g of food) were not affected by treatments ($P > .25$). Carcass weight (124.16 ± 2.09 g) and hot carcass dressing (60.37 ± 0.41%) were not influenced by dietary-CP feeding schedules ($P > .20$). This results confirms the hypothesis that Japanese quail do not requires necessarily consume diet with 24% of CP during complete four weeks fattening period, and is in concordance with reported by Barajas et al. (2004).

During third and fourth weeks quails fed 21% CP-diet ate 13.4% and 13.0% less protein ($P < .01$), respectively than animals consuming 24% CP diet. Over complete experiment, total intake CP decreased linearly as the time in 24% CP-diet was reduced ($P < .01$). Japanese quail fed 24% CP-diet only during their first 14 days of live consumed 12 g of CP less (9.7%) than quails fed 24% CP-diet during 28 days ($P < .01$), and 6.7% fewer than animals fed 24% CP-diet during 21 days. These results coupled with lack effect on feed intake ($P = .29$), outcomes in an enhancement in CP efficiency, that was improved ($P < .05$) in 15% and 11% for 21% CP-diet in relationship with quails ate 24% CP-diet during third and fourth weeks, respectively.

Over complete 28 days fattening experiment, the CP efficiency (g of body gain by each g of CP intake) was 10.2% higher ($P < .01$) in Japanese quail that consumed 14 days 24% CP-diet and remainder 14 days ate a 21% CP-diet (1.73 vs. 1.57 g/g), compared with animals fed 24% CP-diet during 28 days. This result is in concordance with values of protein efficiency equivalents of 1.82 and 1.54 observed by Marks (1971) when fed Japanese quails with diets containing 21 or 24% of CP, respectively.

Boon et al. (2001) explain that CP is required in young Japanese quails to construct new tissues specially muscle and bone that support mostly of growth impetus. Data from this experiment shown that quails increased its weight 20.5 times during fattening period, but the most explosive growth rate is observed during first and second weeks, when quails increased 4.4 and 2.3 times its body mass, respectively, then growth rate is decreased to proportional weekly increments of 1.6 and finally 1.3 times during weeks three and four, respectively. Then agree with Boon et al. (2001), if growth rate is diminished needs of protein for muscle building is decreased too.

Implications

Results of this research suggest that fattening Japanese quail after fourteen days of live decreased its growth impetus and concomitant protein requirements, so that diets containing less than 24% of crude protein could be offered without detrimental effects on growth performance. Proportioning diets containing near of 21% of crude protein during days 15 to 28 to fattening Japanese quail supply enough protein to maintain growth performance, and helps to save close of 10% in protein expenses.

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Table 2. Influence of descending dietary-protein level on performance of growing Japanese quail during cool season (March, 2007).

Item	Treatments [1] Length in 24% CP diet, days			SEM[2]	*P*-value
	14	21	28		
Cages, replicates, n [3]	4	4	4		
Days in trial	28	28	28		
Weight, g					
Day 1	9.8	10.1	9.9	.06	.17
Day 7	43.6	44.7	43.8	.51	.33
Day 14	98.3	100.9	99.0	.97	.19
Day 21	156.6	160.6	156.5	1.30	.08
Day 28	202.6	205.6	203.3	2.00	.57
Weight gain, g					
Week 1	33.6	34.6	33.9	.49	.38
Week 2	54.7	56.3	55.2	.60	.23
Week 3	58.3	59.8	57.5	.59	.16
Week 4	44.1	44.9	46.8	1.49	.66
All 4 weeks	192.7	195.5	193.4	2.00	.60
Feed Intake, g					
Week 1	47.3	49.8	48.7	.68	.08
Week 2	109.2	114.6	11.5	1.74	.15
Week 3	157.4	161.2	157.7	1.84	.31
Week 4	195.0	197.2	197.2	2.81	.82
All 4 weeks	508.9	522.9	515.0	5.83	.29
Feed efficiency, gain g/feed intake g					
Week 1	.71	.69	.70	.01	.32
Week 2	.50	.49	.50	.01	.31
Week 3	.37	.37	.36	.01	.44
Week 4	.24	.23	.24	.01	.48
All 4 weeks	.38	.37	.38	.01	.28
CP Intake, g					
Week 1	11.4	12.0	11.7	.16	.08
Week 2	26.2	27.5	29.8	.42	.15
Week 3 [4, 5]	33.1 [b]	38.7 [a]	37.8 [a]	.42	< .01
Week 4 [4, 5]	40.9 [b]	41.4 [b]	47.3 [a]	.65	< .01
All 4 weeks [6, 7]	111.6 [b]	119.6 [a]	123.6 [a]	1.37	< .01
CP efficiency, gain g/CP intake g					
Week 1	2.96	2.89	2.90	.03	.32
Week 2	2.09	2.05	2.06	.02	.31
Week 3 [4, 5]	1.77 [a]	1.55 [b]	1.52 [b]	.02	< .01
Week 4 [6, 7]	1.12 [a]	1.08 [ab]	0.99 [b]	.03	.02
All 4 weeks [4, 5]	1.73 [a]	1.64 [b]	1.57 [c]	.01	< .01
Mortality, %	8.0	3.5	8.5	3.20	.26
Carcass wt, g [8]	126.9	123.8	121.8	2.09	.24
Carcass dressing, % [8]	60.36	60.78	59.98	0.41	.39

[1] Treatments: 1) First 14 days fed 24% CP-diet and remainder 14 days fed 21% CP-diet; 2) First 21 days 24% CP-diet and remainder 7 days fed 21% CP-diet; and 3) 24 % CP-diet 28 days experiment.
[2] Standard error of the mean.
[3] Mean of 50 quails by cage
[4] Linear trend $P < .01$
[5] CP 24% vs. CP 21%, $P < .01$
[6] Linear trend $P < .05$
[7] CP 24% vs. CP 21%, $P < .05$
[8] Means of 24 quails sample by treatment, constituted by 12 males and 12 females

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EFFECTS OF LEVEL OF SUPPLEMENTAL FLAX OIL ON RUMINAL DISAPPEARANCE OF SOYBEAN MEAL

E. J. Scholljegerdes
USDA-ARS, Northern Great Plains Research Laboratory, Mandan, ND

ABSTRACT[1]: Reductions in forage intake can influence the extent of ruminal degradability of protein and supplemental fats can reduce forage intake. Therefore, the effects of supplemental flax oil on in situ disappearance of soybean meal was evaluated utilizing six ruminally cannulated beef cows (Initial BW = 639 ± 30.2 kg) grazing bromegrass pasture. Cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Supplements were dosed intraruminally at 0730 daily for 28 d. In order to confirm that the level of flax oil fed reduced forage intake, masticate was collected from each cow and TiO_2 was dosed twice daily starting on d 13. Then on d 22, in situ bags (50-µm pore size) containing 5 g of soybean meal were inserted into the rumen and collected at 0, 3, 6, 9, 12, 15, 18, 24, and 48 h after insertion. Effective ruminal degradation was estimated using a combination of this experiment's non-linear regression data and previously determined fluid passage rates. Despite a numerical decline in forage intake ranging from 10,144 to 8,219 g/d as dietary inclusion of flax oil increased, no differences ($P \geq 0.29$) were observed across treatments. Total tract digestion (g/d) and digestibility (% of intake) of DM, N, and NDF did not differ ($P \geq 0.14$) with the exception of total tract NDF digestibility, which tended ($P = 0.06$) to decrease linearly with flax oil inclusion. In situ N disappearance was not different ($P \geq 0.18$) at 0, 3, 6, 9, 12, 15, 18, and 48 h incubation times. However, N disappearance tended ($P = 0.13$) to increase linearly at 24 h. Nitrogen fractions A and B did not differ ($P \geq 0.54$) with level of flax oil. Likewise, no differences ($P \geq 0.26$) in ERD ruminal degradability of N were observed. Overall, supplemental fat fed at levels reported herein were not sufficient to alter the ruminal disappearance of soybean meal when cows grazed brome grass pasture.

Key Words: Fat, In situ, Ruminal degradable protein

Introduction

One of the most notable effects of feeding fat to beef cattle is a depression in dietary intake (Pavan and Duckett, 2008; Scholljegerdes and Kronberg, 2008). A definitive cause of intake depression is unknown, however, research has shown that dietary fats can reduce dry matter intake by lowering ruminal digestion of fiber (Devendra and Lewis, 1974) or by altering hormonal regulation of intake (Choi and Palmquist, 1996). Nevertheless, a decrease in intake with fat feeding may have an effect on ruminal protein digestibility because previous work (Scholljegerdes et al., 2005) has demonstrated that ruminal degradability of protein is altered when intake is restricted.

A reduction in intake may alter the extent of ruminal digestion (Riewe and Lippke, 1969) and a feedstuff's ruminally undegradable protein value (Shadt et al., 1999). Metabolizable protein can often be limited in forages that livestock traditionally graze (Anderson et al., 1988). In addition, cattle fed fats can also be limited in metabolizable protein (Palmquist et al., 1993; Brokaw et al., 2001) due to a reduction in microbial protein production. Therefore metabolizable protein deficiencies may be further exacerbated when fats are fed to grazing cattle. However, it is not known whether or not the depression in forage intake associated with fat feeding is sufficient to cause a change in the proportion of protein degraded in the rumen. The hypothesis for this experiment is that by feeding high levels of fat, forage intake will be depressed and degradable protein content of soybean meal will be altered. Therefore, the objectives of this study were to examine the effects of level of flaxseed oil on the ruminal disappearance of soybean meal.

Materials and Methods

Six ruminally cannulated Angus beef cows (Initial BW = 639 ± 30.2 kg) were used in a completely randomized design to evaluate the effects of supplemental flax oil on in situ disappearance of soybean meal. Cows were allowed to graze a predominately bromegrass pasture starting on June 11, 2008 until July 8, 2008. All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of dietary flax oil inclusion (0, 3, or 6%) was based on predicted total dietary intake for cows as suggested by the NRC (2000). The level of fat supplementation fed in this trial are similar to those known to depress forage intake (Scholljegerdes and Kronberg, 2008). All supplements were dosed intraruminally at 0730 for 28 d.

Although in situ analysis was the primary focus of this experiment, it was important to confirm that our diets indeed influenced intake. Therefore, cattle were allowed 12 d to adapt to diet. Then on d 13, masticate from each cow was collected as described by Brokaw et al. (2001), after which TiO_2 dosing commenced (5 g boluses inserted intraruminally, twice daily). Titanium dioxide was dosed for a total of 10 d with 5 d of adaptation and 5 d of fecal collection occurring at 0730 and 1930. On d 20, just prior to dosing of 200 mL of Co-EDTA into the rumen (Uden et al., 1980), whole ruminal contents were collected and 10

[1] Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA, ARS, Northern Plains Area, is an equal opportunity/ affirmative action employer. All agency services are available without discrimination.

mL of rumen fluid was strained through 8 layers of cheesecloth and acidified with 7.2 N H_2SO_4. Rumen fluid collections occurred at 0, 6, 12, 18, 24, and 36 hr relative to Co-EDTA dosing. Then on d 22, duplicate in situ bags (50-µm pore size, ANKOM Technology, Fairport, NY) containing 5 g of soybean meal were inserted into the rumen and collected at 0, 3, 6, 9, 12, 15, 18, 24, and 48 h after insertion. In situ bags were rinsed and processed as described by Scholljegerdes et al., 2005. On d 28 rumen fluid was collected from each cow for IVDMD determination of masticate and soybean meal.

Masticate, in situ residue, and fecal samples were analyzed for DM and ash (AOAC, 1990), N (Carlo Erba Model NA 1500 Series 2 N/C/S analyzer (CE Elantech, Lakewood, NJ), and neutral detergent fiber (ANKOM 200 fiber analyzer, ANKOM Technology, Fairport, NY). Fecal samples were analyzed for TiO_2 according to the procedures of Myers et al. (2004) using a spectrophotometer (DU -640, Beckman Instruments, Inc., Fullerton, CA). .

Ruminal fluid samples were centrifuged at 20,000 × g for 20 min and a 2.5-mL aliquot was added to 0.5 mL 25% (wt/vol) metaphosphoric acid containing 2 g/L of 2-ethyl-butyric acid (Goetsch and Galyean, 1983). These samples were analyzed for concentrations of VFA as described by Scholljegerdes and Kronberg (2008). Concentration of NH_3 in ruminal fluid was determined using the phenol-hypochlorite procedure of Broderick and Kang (1980). Ruminal fluid Co concentrations were determined by atomic absorption spectroscopy using an air/acetylene flame (Model 3110, Perkin Elmer, Inc., Norwalk, CT).

Intake was estimated from fecal OM output and in vitro OM indigestibility and partitioned between forage and supplement intakes. Protein fractions A and B, as well as protein degradation rate (k_d = %/h) were calculated with the model of Ørskov and McDonald (1970) using the NLIN procedure of SAS (SAS Inst., Inc., Cary, NC). Effective ruminal degradation (ERD) was calculated using the equations of Broderick (1994). Actual fluid passage rate (k_p = %/h) estimates were used to calculate supplement ERD because the supplement would most likely be associated with the fluid phase (Nocek, 1985).

All non-repeated measure data were analyzed with the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). All time course data were analyzed using the MIXED model of SAS as a completely randomized design experiment. Included in the model were the effects of treatment, time, and treatment × time. Autoregression order one was determined to be the most desirable covariance structure according to the Akaike's information criterion. Orthogonal contrasts were used to compare linear and quadratic responses to level of flax oil intake (Steel and Torrie, 1980). There was no treatment × time interactions ($P \geq 0.16$) for any of the variables measured.

Results and Discussion

Forge DM intake did not differ ($P = 0.29$) across treatments (Table 1). This is surprising, due to the fact that when ground flaxseed was fed to provide a diet of 3% total fatty acids for grazing beef cattle (Scholljegerdes and Kronberg, 2007), forage DM intake was significantly reduced. In the current trial, diets consumed were 1.1, 4.5, and 9.0% total fatty acids for 0, 3, and 6% treatments, respectively. Furthermore, Pavan et al. (2007) observed a linear decrease in forage DM intake when corn oil was supplemented at 0, 0.75 and 1.5 g/kg of BW, which equates to a diet that was 2.5, 4.9, and 8.0% total fatty acid. Likewise, total N and NDF intake did not differ ($P \geq 0.14$) across treatment. No differences were observed ($P \geq 0.14$) for DM, N, or NDF digested (g/d). However, there was a tendency for NDF digestibility (% of intake) to decline linearly ($P = 0.06$) with increasing levels of flax oil. A reduction in fiber digestibility has been reported previously when cattle were fed soybean oil (Whitney et al., 2000) and corn oil (Pavan et al., 2007). Total tract DM and N digestibility did not differ ($P \geq 0.20$) across treatments.

Ruminal pH and NH_3 did not differ ($P \geq 0.38$) across dietary treatments (Table 2). Fluid passage rate (%/h) differed quadratically ($P = 0.03$) with cattle receiving 3% flax oil having the lowest values for passage rate. We had expected a linear decrease in forage intake accompanied with a linear decrease in fluid passage rate. Therefore, it is not clear as to why there was a quadratic response in fluid passage rate. Total ruminal VFA concentration did not differ ($P = 0.35$), however, the molar proportion of acetate and propionate differed quadratically ($P = 0.03$). Whereas, proportions of butyrate did not change ($P = 0.25$) with flax oil supplementation. The increase in propionate as oil level increased in the diet is similar to that reported by Kucuk et al. (2004), when lambs were fed increasing levels of soybean oil.

Supplemental flax oil did not affect ($P \geq 0.18$) in situ N degradability (% digested) of soybean meal at 0, 3, 6, 9, 12, 15, 18 or 48 h. Whereas 24 h N degradability tended to be greater (Linear, $P = 0.13$) as flax oil inclusion increased in the diet. Soybean meal N fractions, A and B, did not differ ($P \geq 0.20$) with flax oil inclusion. Degradation rate was not different ($P = 0.20$) despite values ranging from 6.0 to 12.0%/h due in part to large standard errors. Though it is difficult to find published values for in situ degradability of soybean meal in grazing cattle; ruminal N degradability of soybean meal for animals not fed flax oil was similar to that reported by others who fed silage-based diets (Spears et al., 1985; Stern et al., 1994). When ERD was calculated using a k_p that declined linearly as fat levels in the diet increased similarly to those herein (5.7, 6.86, and 6.47 %/h; Scholljegerdes and Kronberg, 2008); no difference ($P \geq 0.26$) was observed (data not shown). However, when actual k_p was utilized (Table 2), ERD tended to differ (Quadratic, $P = 0.08$) with animals consuming 3% flax oil having the greatest ERD compared to 0 or 6% with 6% being greater than 0%. The increased ERD observed in cattle supplemented with 3% flax oil was likely due to a slower fluid passage rate compared to other treatments. Likewise ruminally undegradable protein tended to increase (Quadratic, $P = 0.08$) for cattle fed 3% flax oil compared to 0 or 6%. Palmquist et al. (1993) suggested that cattle fed high-fat diets may have an increased requirement for

ruminally undegradable protein presumably due to a decrease in microbial protein synthesis. Data from our experiment would suggest that deficiencies in ruminally undegradable protein may be further exacerbated due to the tendency for the proportion of protein fermented in the rumen to be greater when fats are fed to grazing cattle.

In conclusion, feeding fats may impact ruminal fermentation patterns and flow kinetics such that ruminal protein degradability is altered. However, the majority of responses reported herein were mere tendencies, therefore more work is warranted in order to better quantify the effect fat has on metabolizable protein supply in grazing cattle.

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Table 1. Effects of soybean meal and increasing levels of flax oil on forage intake and total tract nutrient digestibility in grazing beef cows

	Treatments[1]				Contrast	
Item	0	3	6	SEM[2]	Linear	Quadratic
Forage DM Intake	10144	9483	8219	1050	0.29	0.83
Total DM Intake	10431	10176	9337	1050	0.52	0.84
N Intake	127	119	103	8.4	0.14	0.74
NDF Intake	7515	6990	5839	678	0.18	0.73
Total tract						
DM digested, g/d	7174	6877	6384	800	0.54	0.93
DM digestibility, % of intake	68.8	67.6	68.1	0.96	0.64	0.53
N digested, g/d	97.9	90.7	84.3	8.7	0.35	0.97
N digestibility, % of intake	77.0	76.0	81.6	2.0	0.20	0.26
NDF digested, g/d	5401	4887	3971	507	0.14	0.77
NDF digestibility, % of intake	71.9	70.0	67.7	0.98	0.06	0.91

[1]Cattle grazed a 4.8 ha pasture and were given All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of flax oil fed was based on predicted intake for cows based on NRC (2000) recommendations.
[2]n = 2.

Table 2. Effects of soybean meal and increasing levels of flax oil on ruminal pH, NH_3, fluid passage rate, and volatile fatty acids in grazing beef cows

	Treatments[1]				Contrast	
Item	0	3	6	SEM[2]	Linear	Quadratic
Ruminal pH	5.9	5.7	5.8	0.2	0.87	0.61
Ruminal NH_3, m*M*	4.3	5.0	5.5	0.8	0.38	0.92
Fluid passage rate, %/h	7.5	2.9	10.1	1.2	0.24	0.03
Ruminal total VFA, m*M*	66.4	73.2	67.1	4.8	0.92	0.35
Ruminal VFA, mol/100 ml						
Acetate	59.6	59.4	53.1	0.7	0.01	0.03
Propionate	21.9	22.1	27.4	0.0	0.01	0.03
Butyrate	14.3	14.7	14.2	0.3	0.72	0.25
Isobutryate	1.20	0.94	1.30	0.01	0.35	0.03
Isovalerate	1.9	1.7	2.5	0.2	0.09	0.08
Valerate	1.1	1.1	1.6	0.0	0.04	0.15
Acetate:propionate	2.7	2.7	1.9	0.1	0.01	0.03

[1]Cattle grazed a 4.8 ha pasture and were given All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of flax oil fed was based on predicted intake for cows based on NRC (2000) recommendations.
[2]n = 2.

Table 3. Effects of increasing levels of flax oil on in situ N degradability (% digested) of soybean meal in grazing beef cows

	Treatments[1]				Contrast	
h	0	3	6	SEM[1]	Linear	Quadratic
0	38.5	35.2	36.5	3.9	0.74	0.66
3	44.7	45.9	51.2	6.7	0.38	0.69
6	52.0	45.8	54.3	8.1	0.26	0.55
9	53.0	53.4	68.0	10.7	0.21	0.88
12	63.2	72.4	78.3	6.3	0.18	0.84
15	70.7	76.6	86.4	6.6	0.19	0.82
18	78.6	85.6	90.4	4.8	0.18	0.86
24	80.6	90.4	95.1	4.8	0.13	0.69
48	87.5	92.9	98.4	4.5	0.19	1.0

[1]Cattle grazed a 4.8 ha pasture and were given All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of flax oil fed was based on predicted intake for cows based on NRC (2000) recommendations.
[2]n = 2.

Table 4. Effects of increasing levels of flax oil in situ N fractions and degradability rate of soybean meal in grazing beef cows

	Treatments[1]				Contrast	
Item	0	3	6	SEM[2]	Linear	Quadratic
Fraction A	35.8	32.8	36.1	3.8	0.95	0.55
Fraction B	57.4	63.6	63.1	5.7	0.54	0.66
k_d, %/hr	6.0	8.3	12.0	2.6	0.20	0.84
ERD[3]	61.1	79.7	69.5	4.4	0.28	0.08
RUP[4]	38.9	20.3	30.5	4.4	0.28	0.08

[1]Cattle grazed a 4.8 ha pasture and were given All cows were given 287 g (DM basis) of soybean meal and randomly assigned to one of three treatments that were 0, 3, or 6% dietary flax oil. Level of flax oil fed was based on predicted intake for cows based on NRC (2000) recommendations.
[2]n = 2.
[3]ERD = Effective ruminal degradation = % Fraction A + {% Fraction B · [kd/(kd+kp)]} where kp = 7.5, 2.9, and 10.1%/h for the 0, 3, and 6% treatments, respectively.
[4]RUP = ruminally undegradable protein = 100 – ERD.

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WINTER CEREALS AS A PASTURE-HAY SYSTEM IN MONTANA

A. N. Hafla[1], S. D. Cash[1], L.M.M. Surber[1], J. A. Paterson[1], A. W. Lenssen[2], A. L. Todd[1], M. Huffman[1]
[1]Department of Animal and Range Sciences; Montana State University, Bozeman, MT 59717.
[2]Northern Plains Agricultural Systems Research Unit, USDA-ARS, Sidney, MT 59270.

ABSTRACT: In 2006-2008 'Willow Creek' winter wheat (*Triticum aestivum* L.) and 'Trical 102' triticale (X *Triticosecale* Wttn.) were evaluated, under dryland conditions, for biomass production and forage quality under grazing and haying systems. Grazing enclosures were constructed in uniform sites of the fields. Each enclosure was randomly assigned a treatment (date to be grazed) and a replication (r = 3 in 2006 and 2008, r = 4 in 2007). For the hay-only component, cereals were harvested at the anthesis stage (**A**). For pasture, the cereals were subjected to a single grazing event at three stages of maturity, vegetative (**V**), boot (**B**), and heading (**H**). Ewe lambs grazed plots to approximately 5 cm. Subsequent regrowth was harvested as hay at **A**, and forage yield and quality were measured. Ungrazed forage plots were evaluated for forage yield and quality at each stage of maturity. Hay yields of ungrazed plots at **A** were 4,030 to 13,072 kg/ha for wheat and 8,541 to 12,569 kg/ha for triticale. Grazing wheat at most stages of maturity reduced ($P < 0.05$) subsequent forage yields when regrowth was measured at **A**. Triticale grazed at early **V**, resulted in subsequent forage yields similar to ungrazed triticale ($P > 0.05$), when regrowth was measured at **A**. A single-grazing event of wheat at **V** had available forage yields of 61 to 3,159 kg/ha, and 215 to 601 kg/ha for triticale. Delaying grazing to later stages of maturity resulted in successively greater losses of subsequent forage yield. In a mixed pasture-hay system, total forage availability was impacted by -10 to -29% for wheat and -8 to -28% for triticale, when grazed at **V**. Forage quality was greatest at early **V** and declined throughout maturity. These data indicate that grazing winter cereals in a pasture-hay system at early **V** will maximize total available biomass and forage quality. High forage quality (CP and digestibility) and minimal risk of nitrate toxicity occurred in the mixed pasture-hay system.

Key words: grazing, forage yield, forage quality

Introduction

Livestock producers in Montana are often confronted with the challenge of obtaining affordable feed that provides adequate nutrition to foster animal performance. Annual cereals harvested as hay have become a valuable source of livestock feed and gained popularity as an alternative feed source to traditional hays due to their forage quality and yield (Todd et al., 2007). Cereal forages provide a relatively high protein source for livestock and produce a high total dry matter yield (Stoskopf, 1985).

Winter cereals are rapidly gaining acceptance by producers in Montana as an inexpensive source of livestock hay, and could offer potential as spring pasture. Winter cereals grown in Montana have several advantages when compared to spring seeded cereals. Planting in the fall allows forage harvest to be earlier than spring cereal forage, and can help reduce spring workloads for producers who have livestock and crop enterprises. Additionally, winter cereals generally have greater forage production (Cash et al., 2007). Drake and Orloff (2005) reported that plant stage of maturity at the initiation of clipping affected the amount of subsequent regrowth, under irrigated conditions in intermountain California. No literature is available regarding impacts of livestock grazing on subsequent forage yield of winter cereals in Montana. The objective of this study was to evaluate winter wheat and triticale for biomass production and forage quality under grazing and haying systems, when grown under dryland conditions in Montana.

Materials and Methods

Research Sites and Animals. In a three year grazing study, ewe lambs (*Ovis aries*) were used to evaluate grazing effects on forage yield and quality on plots of winter cereals. Two awnletted, high-yielding cultivars, 'Willow Creek' winter wheat and 'Trical 102' triticale were evaluated. The crops were planted in the fall of the years prior to each study using best management practices for grazing experiments at the Fort Ellis Research and Teaching Farm near Bozeman, MT. Grazing enclosures were constructed in uniform sites of the fields, where wheat and triticale were planted in adjacent strips. Each enclosure was randomly assigned a treatment (date to be grazed) and a replication (r = 3 in 2006 and 2008, r = 4 in 2007). The protocol for this experiment was to subject the crops to a single grazing event at three different growth stages, vegetative (V), boot (B), or heading (H) (Nelson et al., 1998). The first grazing date varied by year, followed by grazing at 14-d intervals to include grazing at B and H. When the ungrazed controls reached anthesis (A), hay harvest occurred. This date varied by year, but is considered to be the forage termination date to preclude excessive soil water depletion in a dryland crop system. Four to eight (depending on forage availability), mixed breed lambs were allowed to graze forage within enclosures to a height of approximately 5 cm, at each date. All experimental animal use was approved by the Montana State University Agricultural Animal Care and Use Committee (MSU-AACUC).

Measurements. Forage biomass was monitored on all treatments throughout the season from V until grain harvest. Total available forage yield was measured by 0.5

m^2 clip samples taken from ungrazed cells at each grazing date and at haying. Clip samples were taken from the inside of plots immediately following grazing to estimate forage utilization at each date. Grazing cell locations were maintained through the season and repeated clip samples were taken from grazed cells at 14-d intervals to evaluate forage regrowth at each grazing date following grazing and at haying. All forage yield estimates were calculated on a DM basis following drying 96 h in a forced air drying oven at 40° C.

Ungrazed forage sampled at each grazing date and at haying were analyzed for forage quality. Forage samples were ground through a 5-mm screen in a Wiley mill and analyzed for 48 h in situ dry matter disappearance (ISDMD) (Van Soest et al., 1991). The unused remainder of each sample was ground through a 1-mm screen and analyzed for CP and nitrate concentration (NO_3-N) (AOAC, 2000).

Statistical Analyses. The experimental design was a completely random design with grazing plots considered the experimental units. Cultivars and grazing treatments (dates) were considered independent variables with forage yield and quality parameters considered dependent variables. Forage biomass and quality variables were analyzed in linear models using ANOVA of Statstix 9.0 software. Means were separated by LSD and considered different at $P < 0.05$.

Results and Discussion

Biomass production. Forage production of ungrazed winter cereals, when measured at forage termination, ranged from 4030 to 13,072 kg/ha for wheat and from 8541 to 12,569 kg/ha for triticale (Table 1). Daily forage dry matter accumulation range from 87 to 246 kg/ha (Table 1). A single grazing event at early V resulted in available forage yields of 61 to 3159 kg/ha for winter wheat and 215 to 601 kg/ha for triticale. Winter wheat grazed at most stages of maturity experienced reduced ($P < 0.05$) forage yields when regrowth was measured at the forage termination date (Table 1). When grazing wheat was delayed until B, forage regrowth was significantly reduced by 48 to 86% when measured at the forage termination date. Triticale grazed at early V had similar ($P > 0.05$) forage yields when regrowth was measured at the forage termination date (Table 1). When triticale was grazed at H, regrowth biomass was reduced by 86 to 92%, when measured at the forage termination date. These results are consistent with Drake and Orloff (2005), who reported that a single clipping event of Trical 102 triticale, occurring at V, produced forage regrowth yield similar ($P < 0.05$) to triticale than had not been clipped. When clipping was delayed to B, forage yield of regrowth was reduced 20% when compared to unclipped triticale (Drake and Orloff, 2005). Delaying grazing of winter cereals to later dates of maturity resulted in successively greater losses in regrowth forage yield.

Total forage biomass in a mixed pasture-hay system ranged from 2865 to 11825 kg/ha for wheat and 6964 to 7502 kg/ha for triticale, when crops were grazed at early V (data not presented). Total forage biomass produced by the pasture hay system was reduced significantly ($P < 0.05$) when grazing was delayed beyond V. When grazing occurred at early V, total biomass production of winter wheat was impacted by -10 to -29%. Total biomass production of triticale was impacted by -8 to -28% when grazed at early V. Grazing winter wheat at B impacted total biomass production of the pasture-hay system by -37 to -51% (data not presented). Similarly, grazing triticale at H impacted the total biomass production by -43 to -47% (data not shown). Data indicate that winter cereals grazed at early V suffer minimal impacts on total forage biomass in a mixed pasture hay system.

Forage Quality and Nitrate Concentration. Digestibility and CP concentrations were highest ($P < 0.05$) at early V, and decreased with maturity (Figures 1 and 2). Cash et al. (2002) recommends that forages with NO_3-N values of 0.2260% and higher be restricted as feed. In 2008, NO_3-N concentrations were between 0.2340 to 0.2434% during the first three dates measured (154 d, 168 d, and 182 d), and then dropped to safe levels at the forage termination date (Figure 2). In 2006 and 2007, NO_3-N concentrations were found to be safe at all dates. Nitrate accumulation is a common problem of cereal forages in Montana, and can affect the feeding value of forages.

Conclusions. These data indicate that grazing winter cereals in a pasture-hay system at early V will maximize total available biomass and forage quality. Digestibility and CP of winter cereals at V was excellent. It will be necessary for livestock producers to consider available biomass, value or pasture and hay, and forage quality and NO_3-N concentrations when using winter cereals in a mixed pasture-hay system in Montana.

Acknowledgments

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Table 1. Forage biomass and regrowth of winter cereals following grazing in a dryland crop system in Montana, 2006 - 2008.

Year, crop and treatment	Date measured (Julian date)						
2006	139 d	153 d	167 d	186 d†		Slope	R^2
Wheat	DM, kg/ha						
Control, ungrazed	61	280^{c}	1537^{c}	4030^{b}		86.8	0.91
Regrowth when grazed at early V (139 d)		125^{c}	708^{d}	2804^{c}		82.9	0.95
Regrowth when grazed at V (153 d)			271^{d}	1873^{d}			
Regrowth when grazed at B (167 d)				1008^{e}			
Triticale							
Control, ungrazed	215	1073^{a}	3344^{a}	8541^{a}		179.5	0.93
Regrowth when grazed at early V (139 d)		621^{b}	2281^{b}	6749^{a}		188.5	0.97
Regrowth when grazed at V (153 d)			364^{d}	2909^{c}			
Regrowth when grazed af H (167 d)				1224^{e}			
P, wheat vs. triticale	*0.1531*	*0.0002*	*0.0000*	*0.0000*			
P, crop x treatment	-	*0.8626*	*0.0388*	*0.0072*			
2007	151 d	165 d	179 d	194 d†		Slope	R^2
Wheat	DM, kg/ha						
Control, ungrazed	3159	4063^{a}	9682^{a}	13072^{a}		247.5	0.94
Regrowth when grazed at early V (151 d)		1535^{b}	4381^{b}	8666^{b}		246.4	0.99
Regrowth when grazed at B (165 d)			1591^{c}	1875^{d}			
Regrowth when grazed at H (179 d)				4509^{c}			
2008	154 d	168 d	182 d	196 d	210 d†	Slope	R^2
Wheat	DM, kg/ha						
Control, ungrazed	1352	2995^{a}	4682^{ab}	10687^{a}	11037^{ab}	193.3	0.92
Regrowth when grazed at early V (154 d)		1253^{b}	2721^{bc}	6478^{b}	8386^{bc}	179.7	0.97
Regrowth when grazed at B (168 d)			2251^{cd}	5326^{bc}	5742^{c}	124.7	0.84
Regrowth when grazed at H (182 d)				574^{d}	830^{d}		
Triticale							
Control, ungrazed	601	1717^{ab}	5142^{a}	9332^{a}	12569^{a}	225.4	0.97
Regrowth when grazed at early V (154 d)		411^{c}	2828^{abc}	6901^{b}	11447^{a}	265.6	0.98
Regrowth when grazed at B (168 d)			1399^{d}	4091^{c}	6598^{c}	185.7	0.99
Regrowth when grazed at H (182 d)				187^{d}	983^{d}		
P, wheat vs. triticale	*0.0187*	*0.0275*	*0.5103*	*0.0203*	*0.0254*		
P, crop x treatment	-	*0.4979*	*0.3766*	*0.0942*	*0.8177*		

† Date of forage harvest for hay was when the ungrazed forage reached the anthesis stage. Mid-July was considered as the target forage termination date in a continuous crop system.

[a, b c, d] Values within a column and year followed by unlike superscript letters differ at $P < 0.05$.

Data transformed by natural log prior to ANOVA; levels of significance and LSD tests were based on transformed data.

Figure 1. Digestibility (%) and crude protein (%) of wheat and triticale, in 2008.

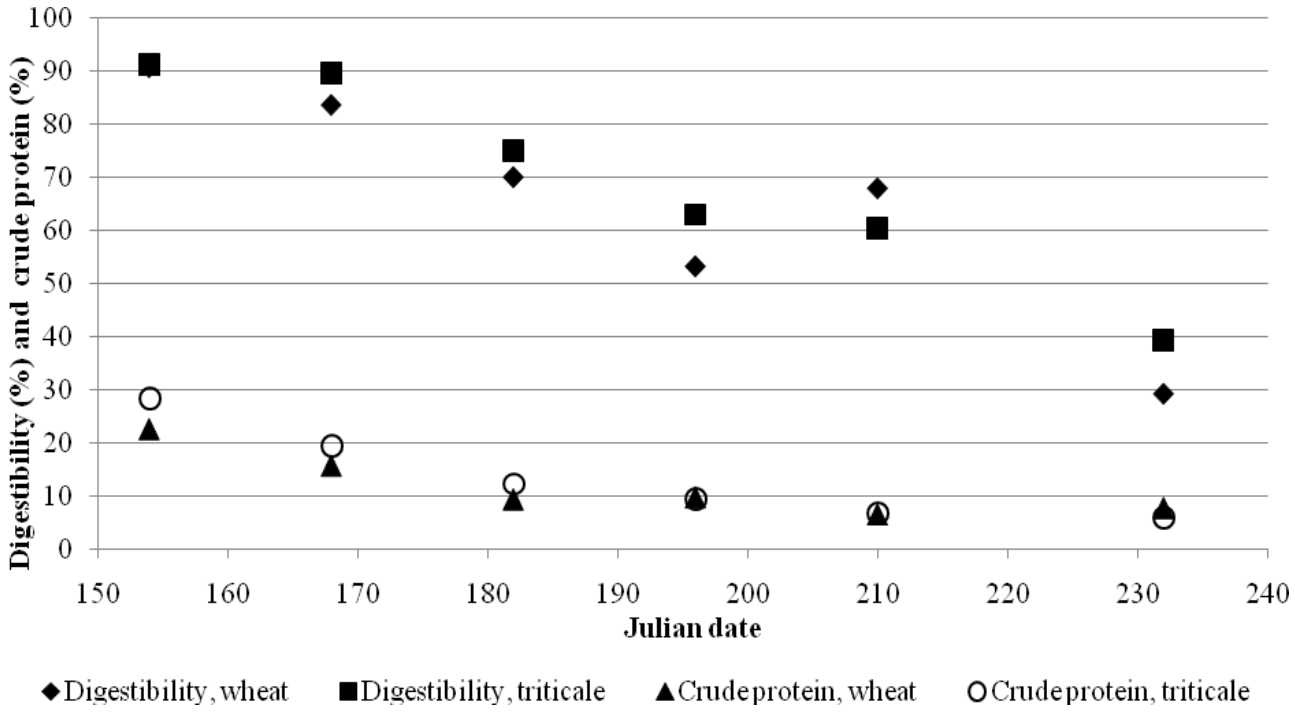
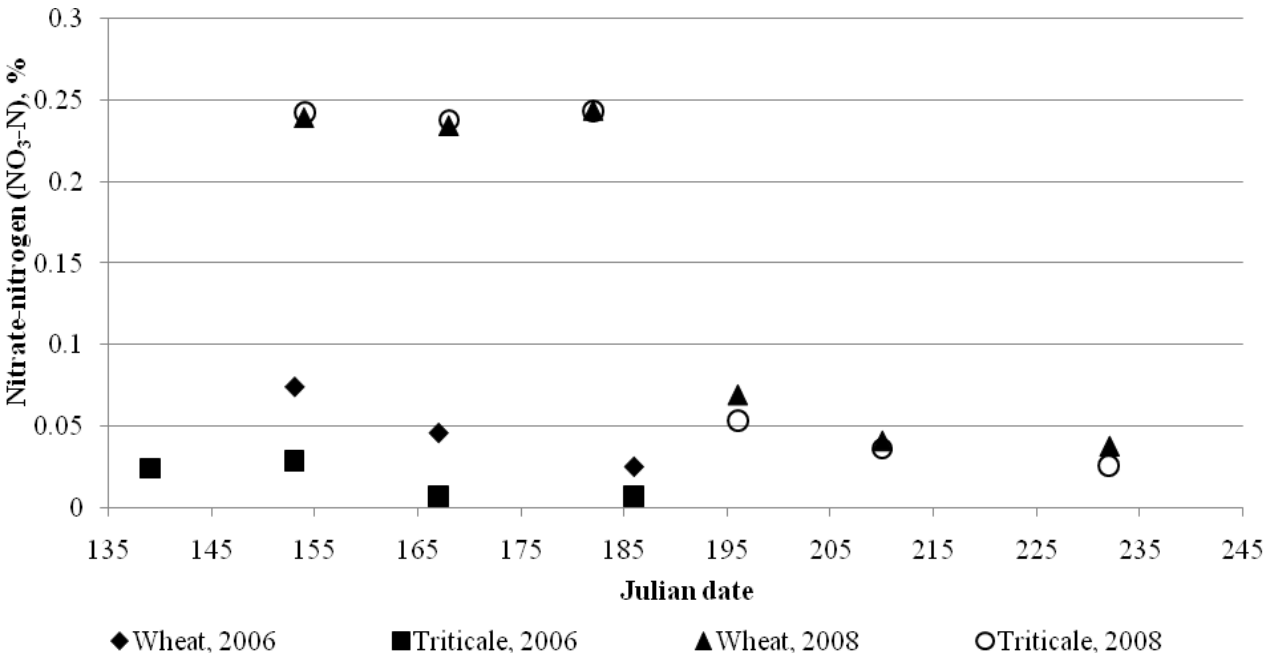


Figure 2. Nitrate concentrations (%) of wheat and triticale, 2006 and 2008.



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SUPPLEMENTAL CORN DRY DISTILLERS GRAINS PLUS SOLUBLES ON PERFORMANCE OF STEERS GRAZING NATIVE RANGE

M. F. Martínez-Pérez*[1], D. Calderón-Mendoza[2,3], F. Loya-Holguin[2,3], C. Murdock[3], A. M. Encinias[3], S. A. Soto-Navarro[1]

Department of Animal and Range Science, New Mexico State University, Las Cruces, NM, USA[1]

Instituto de Ciencias Agrícolas, Mexicali, BC, Mexico[2],

Clayton Livestock Research Center, New Mexico State University, Clayton, NM, USA[3]

ABSTRACT: Medium- to high-quality rangeland forage is low in available energy in relation to its rumen degradable protein content. To complement forage quality, usually energy and phosphorus must be supplemented to cattle grazing medium to high-quality forage. Supplementation with feedstuffs rich in digestible fiber (energy), and phosphorus, such as corn distiller grains plus solubles (DDGS), could alleviate the deficiencies of growing forage. Thus, we hypothesized that supplementation of DDGS to cattle grazing native range during the summer season will alleviate nutritional deficiencies, and will improve cattle grazing performance. To evaluate the effects of DDGS supplementation level on performance of steers grazing native range during the forage growing season, 72 English crossbred steer calves (206 ± 23.6 kg) were used in a 56-d grazing experiment. Steers were blocked by BW into light, medium, and heavy. Each block was divided into 4 grazing groups. Each grazing group (6 steers) was assigned to 1 of 4 DDGS supplementation levels: 1) 0% supplementation (no supplement), 2) 0.2%, 3) 0.4% and 4) 0.6% of BW. Total amount of supplementation per paddock for 7 d was calculated and divided by 3 to determine amount of DDGS to be fed as it was offered 3 times weekly. Supplement intake (0, 0.42, 0.81, and 1.24 ± 0.08 kg/d, for 0, 0.2, 0.4 and 0.6% of BW, respectively), and ADG (0.71, 0.85, 0.91, 0.95 ± 0.06 kg/d for 0, 0.2, 0.4 and 0.6% BW, respectively) increased linearly ($P < 0.01$) with increasing DDGS supplementation level. Levels of DDGS supplementation did not affect ($P = 0.45$) supplement conversion (3.13, 6.53, and 5.37 ± 1.95 kg as-fed supplement/kg of increased BW gain for 0.2, 0.4, and 0.6% BW, respectively). Supplemental DDGS improved performance of steers grazing native range during summer in the Southern Plains.

Keywords: DDGS, grazing, native range, steers

Introduction

Supplementation of grazing animals has long been used to improve grazing production performance. During summer dormancy, or fall and winter months, when forage quality is low, the provision of nutrients to cattle to compensate for deficiencies is practiced often (Caton and Dhuyvetter, 1997). In medium to high-quality forage, such as that during summer growing season, forage is often low in available energy in relation to protein (Pordomingo et al., 1991). Supplementation of energy rather than protein seems to result in favorable responses in BW gain when consuming medium to high-quality forage (Brake et al., 1989). Common sources of supplemental energy vary widely and include grains, readily digestible fiber sources, and high-quality forages (Caton and Dhuyvetter, 1997). Corn supplementation at 0.2% of BW increased forage intake, but greater supplementation levels decreased forage intake (Pordomingo et al., 1991). Several studies involving harvested roughages have demonstrated that the starch contained in grains has detrimental effects on fiber utilization (Fick et al., 1973; Sanson et al., 1990).

With increased demand of ethanol as a biofuel, the availability of cereal grains for livestock production is decreasing (Gottschalk, 2007). Dry distillers grains and condensed solubles (DDGS) are byproducts of fermented cereal grains, corn especially, from the production of ethanol. An increase in the availability of these byproducts is correlated to the rapid expansion of the ethanol industry. These by-products are recognized for being high in readily digestible fiber, protein and phosphorus (Morris et al., 2006). Due to the removal of starch during ethanol production, DDGS' issue regarding starch and forage digestibility are removed (Morris et al., 2005). These characteristics make the product an attractive supplement for medium to high-quality forages. However, little is known about optimum level of DDGS supplementation to cattle grazing medium to high-quality forage and subsequent cattle performance.

The objective of this study was to evaluate the effect of DDGS supplementation on performance of steers grazing native range during summer in the Southern Plains.

Materials and Methods

Animals, Facilities, and Diet. Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. The study was conducted in a pasture located in Dallam County, TX, 64 km east from the Clayton Livestock Research Center in Northeastern New Mexico. Sideoats (*Bouteloua curtipendula*) and bluegrama (*Bouteloua gracilis*) were the major plant species on the study site. Other important forage species in the area included, old world bluestem (*Andropogum gerardii*), galletagrass (*Hilaria jamesi*) and buffalograss (*Buchloe dactyloides*). Seventy-two English crossbred steer calves (206 ± 23.6 kg) were blocked by BW into light, medium, and heavy. Each block was divided into 4 grazing

groups (6 steers per group) per pasture. Each pasture was approximately 25.5 ha total, and all pastures were divided into 4 paddocks (6.4 ha) each with electric fencing to allow rotation of steers within pasture. Steers were adapted to DDGS for 2 weeks. During adaptation, steers received sudan hay ad-libitum and 100 g/head of DDGS daily. Once transferred to experiment location, steers were allowed free access to water, 1-580 L tub per paddock. Supplemental DDGS was offered in feeders, 1 placed in each paddock according to treatment. Total amount of supplementation per paddock for 7 d was calculated and divided by 3 to determine amount of DDGS to be fed as it was offered 3 times weekly at 0900.

Design and Treatments. The experimental design was a randomized complete block design consisting of a 56-d grazing study. Treatments consisted of 4 DDGS supplementation levels: 0% (no supplement), 0.2%, 0.4% or 0.6% of BW. The total amount of supplement was consumed. Therefore, supplement intake was equal to supplement offered. Steers were weighed at beginning and at end of the experiment to measure weight gain and supplement conversion (kg of DDGS as fed • kg of increased BW gain). The amount of DDGS offered was based on initial BW.

Sample forage clippings were taken from the pasture at 3 time intervals, at the beginning of the experiment (d 0), at d 30, and at end of trial (d 56) using the rapid assessment method (0.093m^2 ring). Each paddock was divided in 3-100 m equally spaced transects (1/3 from beginning, 2/3 at middle, and 3/ at end of paddock) and obtaining 4 samples (g DMB/m^2) at 12.5 m apart within transect. A total of 432 samples were taken from the pasture, 144 samples per interval, and 12 samples per paddock. Clippings were dried at 55°C in a forced-air oven. Available standing crop within each block was determined with the use of clippings. The biomass availability was 858, 988, and 1214 kg/ha for light, medium, and heavy block, respectively.

Laboratory Analysis. Clipping samples and DDGS were analyzed for DM, ash, NDF, CP, and ether extract to determine nutrient composition.

Statistics. Data were analyzed using the Mixed procedures of SAS (SAS Inst. Inc., Cary, NC). The model included effects of treatment as fixed effects and block as random effects. Orthogonal contrasts were conducted for linear, quadratic, and cubic effects of DDGS supplementation level.

Results and Discussion

The effect of supplemental corn DDGS on performance of steers grazing native range is shown on Table 1. Supplemental intake and ADG increased linearly ($P < 0.01$) with increasing DDGS supplementation level. Similar results were found by Morris et al. (2005), where yearling steers grazed Sand hill range and supplemented at 0, 0.26, 0.57, 0.77, and 1.03% of BW; a linear increase in ADG with increasing level of DDGS supplementation was found. This can be due to the increased amount of readily digestible fiber in DDGS, as well as high CP forage. Therefore, CP would not be a limiting nutrient for the animal. Vanzant et al. (1990) found that at low grain supplementation levels (0.4% of BW) does not affect on forage intake when CP is not limiting, and thus addition of grain to diet may increase total DE intake. Similar results with low-quality bluestem hay verified these findings (Brake et al., 1989).

Supplement conversion (additional BW gain per kg of DDGS supplemented) was not affected ($P = 0.45$) by DDGS supplementation level. The supplement conversion values were 3.13, 6.53, and 5.37 ± 1.95 kg as-fed supplement/kg of increased BW gain for 0.2, 0.4, and 0.6% of BW, respectively. The supplement conversion values observed in this experiment are in close agreement with those observed when high-fiber by-products feeds were supplemented to stocker cattle grazing wheat pasture (Horn and McCollum, 1987).

Implications

Supplementation of energy to cattle grazing medium to high-quality forage can improve livestock production with regards to BW gain over time. Our findings suggest that supplemental DDGS improved performance of steers grazing native range during summer in the Southern Plains, and the conversion of supplemental DDGS to BW gain seems highly acceptable. Therefore, DDGS are a viable alternative as a supplement for growing cattle grazing medium to high-quality native forage in the Southern Plains.

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Table 1. Effect of supplemental corn DDGS on performance of steers grazing native range

	DDGS supplementation level, %[a]					Contrast[b]		
Item	0	0.2	0.4	0.6	SE[c]	L	Q	C
Initial BW, kg	203.05	209.35	205.61	204.39	23.6	0.98	0.34	0.47
Final BW, kg	243.03	257.16	256.71	258.05	25.05	.002	.01	0.09
ADG, kg/d	0.71	0.85	0.91	0.96	0.06	0.006	0.31	0.73
Supplement intake, kg/d	0	0.42	0.82	1.24	0.09	<0.01	1.00	0.83
Supplement conversion[d]	-	3.13	6.53	5.37	1.95	0.45	0.38	-

[a]DDGS supplementation was offered at: 0 (no supplement), 0.2, 0.4, and 0.6% of BW.
[b]Probabilities for contrasts: linear (L), quadratic (Q), and cubic (C).
[c]SE with n = 3.
[d]Supplement conversion kg of as-fed DDGS supplement per kg of increased BW gain.

Adaptation of warm-season annual legumes for forage in southeastern New Mexico

D. Calderon-Mendoza, A. Islas, M. F. Martinez-Perez, J. Armendariz, F.E. Contreras-Govea, S. Angadi, M. Marsalis, L. Lauriault, S. A. Soto-Navarro

Six warm-season annual legumes were used in a completely randomized block design (4 replicates per legume) to assess the adaptation to Southeast New Mexico and to determine forage yield potential and forage nutritive value. The legumes used were lablab (*Lablab purpureus L*, cv Rio verde), lablab (*Lablab purpureus L*, cv Rongai), cowpea (*Vigna unguiculta L*, cv Iron and Clay), cowpea (*Vigna unguiculta L*, cv Catjang), trailing wildbean (*Strophodtyles helvula L*, cv TX-00H1), and smooth-seeded wildbean (*Strophodtyles leiosperma,* cv TX-00-L1). The land was divided in 4 blocks with each block containing each legume. Each legume was sown in a 1.2 x 4.5-m plot size, 4 rows/plot, and 0.3 m separation between rows. Each experimental unit had a 1.5-m separation. Soil was a Reagan clay loam. Each plot was fertilized with 220 kg P_2O_5/ha and 180 kg K_2O/ha, based on recommendations for alfalfa. Nitrogen was not applied on any plot. Legumes were irrigated 3 times before harvest. Beans were harvested 1 time in September, 82 d after planting. All plots were hand clipped at a stubble height of 3.8 cm. Smooth-wildbean did not germinate on any of the 4 replicates and trailing wildbean had very low germination. On the other hand, lablab cvs rongai and rio verde, and cowpea cv. Iron and clay were the legumes with greater ($P < 0.05$) DM yield. Lablab rongai and rio verde produced similar yield, but an average 0.5 ton/ha more ($P < 0.05$) than cowpea cv. Iron and clay and 2.0 ton/ha more ($P < 0.05$) than cowpea cv. cadjan and trailing wildbean. All 5 legumes had good quality characteristics; CP concentration ranged from 18 to 22%, NDF concentration from 26 to 34%, ADF concentration from 23 to 28%, and NE for lactation (NE_L) from 1.52 to 1.63 Mcal/kg. In vitro DM digestibility was lower for trailing wildbean (38.2 ± 0.01 %) than the other legumes which ranged from 45.2 to 48.6 (± 0.01) %. Considering that beans were sown in June and only harvested 1 time, the DM yield can be considered acceptable for this region. Results suggest that these warm-season annual legumes have potential for Southeast New Mexico to fit in the forage production system or crop rotation system.

Key words: Annual, in vitro, legumes, warm-season

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EFFECT OF WINTER SUPPLEMENTATION STRATEGY ON BODY WEIGHT AND CONDITION CHANGES

F. W. Harrelson, S. L. Lodge-Ivey, S. H. Cox, R. L. Dunlap, C. A. Löest, and M. K. Petersen

New Mexico State University, Las Cruces, NM

ABSTRACT: Recent research indicates that utilizing a self-fed protein supplement (characterized by low ruminal protein degradability) mixed with a complete salt mineral mix may be a nutritional and economic alternative to traditional hand-feeding. A two year study was designed to evaluate BW and BCS change in late gestation cows when offered a traditional 36% CP (35% UIP) hand-fed cottonseed meal (CSM) based supplement (CON) versus a 33 % CP (60% UIP) self-fed (SSP) mineral-fishmeal supplement. Year 1 utilized 128 mature (3.5 - 10.5 yr of age, 539 ± 5 kg BW) Angus and Angus crossbred cows, whereas in year 2, 149 mature (3.5 - 11.5 yr of age, 530 ± 4 kg BW) Angus and Angus crossbred cows were used. Cows were randomly assigned to 1 of 4 replicated native pastures; pastures were randomly assigned to receive either CON or SSP treatment. The CON treatment was fed at 454g/d ($9.69/hd yr 1 and $9.31/hd yr 2) and distributed 3 d/wk. The SSP treatment consisted of a self-fed 50% fishmeal and 50% mineral mix, with a targeted intake of 113g/d (yr 1 = $20.66/hd; yr 2 = $20.77/hd). Supplementation was offered from mid-December through mid-February (yr 1 = 61 d, yr 2 = 50 d). Upon initiation of calving, prepartum supplementation was discontinued and all cows were supplemented similarly (36% CP cube at 908g/d, fed 3 d/wk). No interaction between supplement strategy and year was observed ($P > 0.25$) for BW or BCS. Supplementation strategy had no effect on BW ($P = 0.57$) or BCS ($P = 0.50$). A significant ($P = 0.09$) effect of year was observed for BW change (4.1 kg for yr 1 and -16.8 kg for yr 2). Also, year affected ($P < 0.01$) the change in BCS, with a -0.67 change in year 1 and a -0.21 change in year 2. This study showed that pregnant cows supplemented with either a self-fed fishmeal-mineral or the traditional CSM based supplement experienced similar changes in winter body weight and condition.

Keywords: beef cattle, protein supplementation, winter range

INTRODUCTION

Winter supplementation is often a crucial part of managing spring calving herds on western rangeland. A major reason is due to the mature forage being low in protein and possibly energy (Krysl et al. 1987; Soder et al. 1995). Supplemental protein has been shown to be effective at maintaining BW (DelCurto et al. 1990); however supplementation may be labor intensive and expensive (Melton and Riggs, 1964). Recently, a supplement based on feeding small levels of ingredients high in UIP combined with minerals and salt was shown to both maintain BW and BCS (Sawyer et al. 2005), as well as maintain ruminal function on low quality forage diets (Sawyer et al. 2000). The objective of this study was to compare a self-fed small supplement strategy, utilizing fishmeal as a UIP source, to a conventional hand-fed (cottonseed meal based) supplement on winter BW and condition changes.

MATERIALS AND METHODS

This study was conducted for 2 consecutive winters (2007 and 2008) at the New Mexico State University Corona Range and Livestock Research Center, Corona, NM. The average elevation at the study site is 1,900 m. Annual precipitation averages 370 mm, with 70% of this precipitation occurring between May and October (Torell et al. 2008). This study contributes to a larger integrated systems approach research project evaluating maternal nutrition on calf health and performance. All animal handling and experimental procedures were in accordance with the New Mexico State University Institutional Animal Care and Use Committee guidelines.

Year 1

The first year of research utilized 128 mature Angus and Angus crossbred pregnant cows ranging in age from 3.5 to 10.5 yrs (539 ± 5 kg BW). Pregnancy was confirmed via rectal palpation prior to trial initiation. Cows were weighed 1 month prior to trial initiation and were randomly assigned by BW to 1 of 4 replications. Each replication was randomized to replicate native pastures which were randomly assigned 1 of 2 treatments. Treatments consisted of 1) traditional 36% CP hand-fed supplement (CON) or 2) NMSU self-fed small supplement package (SSP). The CON supplement was a cottonseed meal based range cube with 35% of the CP being UIP. Composition was 57% cottonseed meal, 21% wheat middlings, 10% soybean meal, 9% molasses, 1.2% urea and fortified with trace minerals and vitamins. The CON supplement was fed at a rate of 454 g/d delivered 3 d/wk. Prices for the supplement varied between years due to fluctuations in supplemental ingredients. For yr 1, CON was purchased at $15.90 / 45.4 kg, whereas in yr 2 it was purchased at $18.79 /45.4 kg.

The SSP supplement (NMSU small package supplement) was formulated to contain 33% CP (60% UIP), and composed of 50% fishmeal, 33 % minerals, and 17% salt. The SSP supplement was formulated for a target consumption of 113 g/d. Winter supplementation was ended two weeks prior to the expected start of parturition within the herd (mid-February). Supplementation after

parturition was similar for all cows (CON fed at 908 g/d fed 3 d/wk).

Cows were weighed and BCS assigned at trial initiation in December, midway through supplementation period (January), and the end of supplementation in February. Condition scores were assigned visually and via palpation by a trained technician using a scale from 1 (emaciated) to 9 (obese).

Year 2

Research in year 2 utilized 149 mature Angus and Angus crossbred cows ranging in age from 3.5 to 11.5 years, and weighing 530 ± 4 kg. Methods for yr 2 were designed to be identical to those utilized in yr 1.

Economic Evaluation

A basic economic comparison was conducted to compare financial differences between the two supplement strategies. The analysis included the input costs associated with the supplement, hours necessary for mixing (SSP), delivery hours, and the cost of fuel for supplement delivery. Labor cost associated with mixing and delivery were assumed to be $7.00/hr, therefore the total number of mixing and delivery hr were multiplied by $7.00 and then divided by the number of cows. For mixing, 1 hr was necessary for each SSP batch, and 1.5 hr was allocated for delivery of each batch. CON was premixed, therefore mixing time was 0, while delivery was 3 hr for each d supplement was provided. Gasoline usage was 7.58 L/delivery for both SSP and CON; gasoline was inputted at $2/3.79 L in yr 1 and $2.50/3.79 L in yr 2.

Due to technician errors cow consumption levels of SSP were allowed to exceed targeted levels as reported by Sawyer et al. (2000). Therefore SSP deliveries were double the predicted, therefore an assumptive economic analysis was also conducted to evaluate the differences had SSP been delivered once/wk compared to the twice/wk as observed, though this assumptive analysis does not adjust for the over consumption (2× target) of SSP.

Statistical Analysis

Data for both years were analyzed as a completely randomized design utilizing the MIXED procedure of SAS (SAS Inst., Cary, NC) with the experimental unit being pasture. The model included the fixed effects of year, supplemental treatment and their interaction.

RESULTS AND DISCUSSION

Supplement consumption rate, duration, and total consumption for each supplement are presented in Table 1.No significant year×supplement interaction ($P > 0.25$) was observed, therefore only the main effects of supplement and year will be presented. Supplement effects on body weight and condition change are presented in Table 2. Initial BW was similar ($P = 0.99$) for SSP and CON as was interim BW ($P = 0.93$) and final BW ($P = 0.60$). Supplement also showed no effect on initial BCS ($P = 0.86$), interim BCS ($P = 0.25$), or final BCS ($P = 0.51$). Winter BW change ($P = 0.57$) was not influenced by supplement nor was change in BCS ($P = 0.50$).

Table 1. Feeding rate, total consumption, and duration of supplementation for two winters.

Item	SSP	CON
Year 1		
Consumption rate, g/d	295	454
Duration, d	61	61
Total intake, kg	18.0	27.7
Year 2		
Consumption rate, g/d	346	454
Duration, d	50	50
Total intake, kg	17.3	22.7

Table 2. Effect of supplement on winter body weight and condition.

Item	SSP	CON	SEM	*P*-Value[1]
Body Weight Response				
Initial BW, kg	535	535	2	0.99
Interim BW, kg	532	531	6	0.93
Final BW, kg	525	531	7	0.60
BW Change, kg	-9.3	-3.4	6.6	0.57
Body Condition Response				
Initial BCS	5.0	5.0	0.1	0.86
Interim BCS	4.5	4.7	0.1	0.25
Final BCS	4.6	4.5	0.1	0.51
BCS Change	-0.4	-0.5	0.1	0.50

[1]Protected F-statistic for the effect of supplement.

Yearly effects on BW and condition changes are represented in Table 3. Initial BW was significantly affected ($P = 0.02$) by study year with cows entering year 2 lighter than entering year 1 (530 vs. 538 ± 2 kg, respectively). Consequently, year also affected both interim BW ($P = 0.04$) and final BW ($P = 0.05$) as cows were lighter throughout the study in year 2 compared to year 1. Similarly, initial BCS was affected by year ($P < 0.01$) with cows in year 2 beginning lower than those in year 1 (4.8 and 5.1 ± 0.03, respectively). Unlike the BW response, year did not influence interim ($P = 0.65$) or final ($P = 0.20$) BCS. Overall changes in BW ($P = 0.09$) and BCS ($P < 0.01$) were significantly affected by year. Body weight change was -17 kg for year 2, while the change during year 1 was +4 kg. The year 2 change in BCS was -0.2 whereas it a -0.7 change was seen in year 1.

Table 3. Effects of year on winter body weight and condition.

Item	Yr 1	Yr 2	SEM	*P*-Value[1]
Body Weight Response				
Initial BW, kg	539	530	2	0.02
Interim BW, kg	543	519	6	0.04
Final BW, kg	543	514	7	0.05
BW Change, kg	4.1	-16.8	6.6	0.09

Body Condition Response				
Initial BCS	5.2	4.8	0.1	<0.01
Interim BCS	4.6	4.6	0.1	0.65
Final BCS	4.5	4.6	0.1	0.20
BCS Change	-0.7	-0.2	0.1	<0.01

[1]Protected F-statistic for the effect of year.

Table 4 shows the economic comparison between supplements at the delivery frequency within this study. For both the CON and SSP, the largest expense was the cost of the supplement, however the transportation costs were almost equal to supplement costs in yr 1 for the CON. Comparing the actual expenses to those projected with SSP delivery once/wk, yr 1 total cost of SSP would be reduced from $27.38 to $23.68, whereas in yr 2 the cost would be reduced from $24.07 to $22.87. The projected reduction in deliveries for yr 1 would be from 20 to 10, and in yr 2 from 11 to 7, which would account for the decrease in total costs. It is important to note that since the cows consumed over twice the target intake, this analysis means vary little in comparing supplement costs.

Table 4. Actual economic comparison between supplemental treatments.

	Year 1		Year 2	
Item	CON	SSP	CON	SSP
Deliveries	26	20	21	11
Supp cost, $/45.4 kg	15.90	52.00	18.79	54.49
Supp cost, $/cow	9.69	20.66	9.31	20.77
Mixing cost, $/cow	0.00	2.19	0.00	1.03
Delivery cost, $/cow	8.53	3.28	5.88	1.84
Gasoline cost, $/cow	1.63	1.25	1.40	0.73
Total cost, $/cow	19.85	27.38	16.59	24.07

The results of this study support previous research conducted at NMSU utilizing either blood and feather meal (Sawyer et al., 2005) or corn gluten meal (Mulliniks et al., 2007) as the UIP source within a self-fed supplement. Both of these studies compared the self-fed supplement to a hand-fed 36% CP hand-fed supplement. The results from these previous studies suggested that the self-fed supplement did not affect BW or BCS change. Unlike Sawyer et al. (2005) our feed costs for the SSP treatment was higher than the CON, most likely due to the over consumption of the SSP.

IMPLICATIONS

The use of a fishmeal-mineral mix self-fed supplement resulted in similar BW and BCS changes compared to an oilseed based, hand-fed supplement. Feed costs were slightly lower for the hand-fed supplement, though this advantage may not be observed when compared on a nutrient basis.

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ADAPTATION OF FALL SOWN MEDIC, PEA, VETCH, AND LENTIL TO THE 2007-2009 CLIMATE OF THE HIGH PLAINS OF WYOMING.

C.L. Loehr, J.J. Nachtman, J.T. Cecil, S.I. Paisley, and J.M. Krall
University of Wyoming, Laramie, WY

ABSTRACT: The objective of this study is to identify new winter annual forage legume crop options for the central high plains as part of an ongoing irrigated annual legume winter survival assessment at the University of Wyoming Sustainable Agriculture Research and Extension Center (SAREC) in southeastern Wyoming. As a potential 'ley' species, medic shows promise, as Laramie medic (Medicago rigidula) had 95% winter survival, and Medicago phrygia had 98% survival in a replicated trial in 2008. In separate trials conducted in 2008 and 2009, four replicates of each large seeded legume were compared to Laramie medic for winter hardiness in a randomized complete block design. Winter survival was measured on 3 April, 2008, with Common Austrian winter pea (39% survival) comparable to Laramie medic (37 % survival). In the same trial, mean survival was 48, 1, 0, 0, 0, and 11% respectively for Common hairy, Namoi wooly pod, Rasina and Morava vetches, and Indian Head and Toni lentils. On 4 March 2009, mean survival for Austrian winter pea and Common hairy vetch was 100%. Survival of Namoi wooly pod vetch and Toni lentil were 88.75% and 90% respectively. Laramie medic was lower, at 55% survival. Morava vetch, Indian head lentil, and Rasina vetch showed no winter survival. Forage production, and stand forage quality will be evaluated during the 2009 growing season. As mean temperatures rise, water availability declines, and input costs increase, annual legume forages will become increasingly important components of lower input crop rotation systems.

Key words: Annual forage, Medic, Ley cropping.

Introduction

The average temperature in Laramie, WY has increased 0.8^{o} C over the last century, according to the EPA. Projections show that by 2100 average temperature in Wyoming could increase by 2.2^{o} C in spring and fall, 2.8^{o} C in summer, and 3.3^{o} C in winter. The EPA forecasts that climate change could increase wheat yields by 35-48% and reduce corn yield by about 13%. Climate change requires the assessment of plant materials for their suitability to these milder winters and hotter summers. Ley farming systems utilizing legumes have been in use in Australia since the mid 1930's because of their positive effects on cereal crop yields (Puckridge and French, 1983). These systems incorporate a legume pasture/wheat crop rotation. The benefits of such a rotation include increased soil fertility and soil structure and reductions in weed and disease problems (Walsh et al, 2001). It is believed that southeastern Wyoming has a climate and soil type which will support and benefit from ley farming systems. Wyoming wheat producers primarily use a wheat fallow rotation in which the land sits dormant every other year and thus earns no profit. It is our belief that these producers would benefit from a ley farming method in which wheat was rotated with a legume. The nitrogen added to the soil by the legume would be especially beneficial considering the currently high cost of nitrogen fertilizer.

The objective of this study is to identify new winter annual forage legume crop options for the central high plains as part of an ongoing irrigated annual legume winter survival assessment in southeastern Wyoming.

Materials and Methods

Nine different legumes were tested for winter survival at the James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC) near Lingle Wyoming. Varieties tested were: Laramie Medic (*Medicago rigidula*), Austrian Winter Pea (*Pisum sativum* L.), Common Hairy vetch (*Vicia villosa* Roth), Namoi Wolly Pod vetch (*Vicia villosa*), Rasina vetch (*Vicia sativa*), Morava vetch (*Vicia Sativa*), Indian Head lentil (*Lens. culinaris*), and Toni lentil (*Lens. Culinaris*). Namoi Wooly Pod vetch, Rasina vetch, Morava vetch, and Toni lentil are all Australian species being tested for their suitability to southeastern Wyoming. The other varieties are common to the United States, but not necessarily to southeastern Wyoming. The eight legumes were planted on August 28, 2007 and October 2, 2008 to be evaluated for winter survivability. Each variety was replicated four times in a randomized complete block design. Seeds were inoculated prior to planting. Plots measured 1.5 meters by 6.1 meters. Row spacing measured 35.6 centimeters. Winter survival evaluations were taken on April 3, 2008 and March 4, 2009. Harvesting of the first year's plots took place on May 5, 2008. The second year's plots have yet to be harvested Two meters of row cover was harvested by hand for comparison of forage production. The entire experiment was also duplicated in the spring of 2008 and 2009 to evaluate each species' suitability as a summer crop. Planting took place on April 4, 2008 and May 19, 2009. Harvesting took place on July 1, 2008. The second year's plots will be harvested later in the year. Statistical

analysis was done on the data using the ANOVA procedure in SAS (SAS, Cary NC).

A separate experiment was also conducted which compared Phrygia medic (*Medicago phrygia*) against Laramie medic for suitability to be grown in southeastern Wyoming. Phrygia medic has recently been brought from Australia, and this is its first trial in Wyoming. Six one meter square plots were planted of each variety in the fall of 2007. The number of live plants were counted on November 11, 2007 and again on April 13, 2008 to assess winter survivability. Differences were detected using the PROC GLM procedure in SAS.

Results

When percent row cover was measured for 2008 on April 13, it was discovered that only five of the varieties were showing signs of surviving the winter. These varieties were Toni lentil, Austrian winter pea, Laramie medic, Hairy vetch, and Namoi wolly pod vetch. The percent row cover is shown in Table 1. Hairy vetch had the highest survival at an average 48% row cover followed by Austrian winter pea at 39% and Laramie medic at 37%. All other varieties were far below these averages. On May 30, 2008 when the plots were harvested, it was found that only three varieties Hairy vetch, Laramie medic, and Austrian winter pea survived the winter. Laramie medic was the highest producer averaging 43.75 g of dry matter per two meters of row cover harvested. It was followed by Hairy vetch at 30 g and by Austrian winter pea at 27.5 g as shown in Table 2.

When the row cover readings for 2009 were taken on May 4, it was found that again, only five of the varieties survived the winter, as shown in Table 3. Austrian winter pea and Hairy vetch both averaged 100% row cover. Toni lentil averaged 90%, Namoi wolly pod vetch averaged 88.75%, and Laramie medic averaged 55% row cover.

In the summer of 2008, forage production was collected from seven of the eight varieties. All varieties were planted and harvested with the exception of Laramie medic as it only functions as a fall sown legume. Dry matter averages were taken two meters of row cover harvested from all 7 of the forages. As shown in Table 4, Morava vetch and Austrian winter pea yielded 251.74 g and 247.66 g DM respectively. Forage production for Rasina vetch followed closely at 218.18 g. Indian head lentil and Namoi wolly pod vetch were intermediate at 118.39 g and 173.27 g. Toni lentil at 81.65 g and Hairy vetch at 76.20 g were both low producers as compared to the other varieties. In the Medic trial, both varieties survived the winter. When the average number of plants per plot were compared between the two, Laramie medic was found to be superior ($P < 0.05$), averaging 164.83 plants per plot in the fall and 156 in the spring. Phrygia medic averaged only 51.83 plants per plot in the fall and 50.83 in the spring. Phrygia medic was also found to be devoid of root nodules, meaning that it was not fixing any nitrogen. We assume that this was due to it not being compatible with the inoculants used to treat the seed prior to planting. This is shown in Table 5.

Table 6 shows the nutrient analysis that was performed by SDK Laboratories (Hutchinson, KS) on all varieties that survived the winter, including both Medic varieties.

Discussion

After testing all eight varieties for winter survival and production, it would appear that only Austrian winter pea, Laramie medic, and Hairy vetch are suited to be used as winter legumes in a ley farming system in southeastern Wyoming. However, this year's data seems to suggest that Toni lentil and Namoi wolly pod vetch may also be possibilities. Additional research may be needed to make a definite conclusion. Economic research may also be needed to decide if ley farming is indeed a profitable option for Wyoming producers.

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Table 1. Average spring 2008 percent row cover, fall-sown.

Variety	Value
Austrian Winter Pea	39
Laramie Medic	37
Common Hairy Vetch	48
Namoi Wolly Pod Vetch	1
Rasina Vetch	0
Morava Vetch	0
Indian Head Lentil	0
Toni Lentil	11

Table 2. Average summer 2008 forage production and dry matter analysis in grams\2 m row cover fall-sown.

Variety	Value
Austrian Winter Pea	27.5
Laramie Medic	43.75
Common Hairy Vetch	30
Namoi Wolly Pod Vetch	0
Rasina Vetch	0
Morava Vetch	0
Indian Head Lentil	0
Toni Lentil	0

Table 3. Average spring 2009 percent row cover fall-sown.

Variety	Value
Austrian Winter Pea	100
Laramie Medic	55
Common Hairy Vetch	100
Namoi Wolly Pod Vetch	88.75
Rasina Vetch	0
Morava Vetch	0
Indian Head Lentil	0
Toni Lentil	90

Table 4. Average summer 2008 forage production and dry matter analysis in grams\2 m row cover spring-sown.

Variety	Value
Austrian Winter Pea	247.66
Common Hairy Vetch	76.20
Namoi Wolly Pod Vetch	173.27
Rasina Vetch	218.18
Morava Vetch	251.74
Indian Head Lentil	118.39
Toni Lentil	81.65

Table 5. Medic variety comparison average number of plants\m^2 plot.

Variety	Fall	Spring
Phrygia Medic	51.83	50.83
Laramie Medic	164.83	156

Table 6. Nutrient analysis of fall 2007 sewn varieties which survived winter.

Variety	CP	TDN	RFV
Laramie Medic	18.9	58.3	148
Phrygia Medic	18.9	64.0	150
Austrian Winter Pea	31.8	73.3	245
Common Hairy Vetch	25.5	63.8	168

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EFFECT OF CALF SEPARATION DURING THE 12-H INTERVAL BETWEEN TWO PROSTAGLANDIN INJECTION IN BEEF COWS SYNCHRONIZED FOR OVULATION USING THE 5-D CO-SYNCH + CIDR PROTOCOL

Priest[1], M.K., R.K. Peel[1], W.D. Whittier[2] and J.C. Whittier[1]

[1]Colorado State University, Fort Collins; [2]Virginia Tech University, Blacksburg

ABSTRACT: Our objective was to determine the effect on fixed-time artificial insemination (TAI) pregnancy rates when separating the calf from its dam during the 12-h interval between the two PG injections following CIDR removal in beef cows synchronized with the 5-d CO-Synch + CIDR protocol. Our desire was to simplify the application of this protocol by avoiding the need to put the calves back with their dams then separate them again for the second injection. This experiment was conducted at two separate ranches during the spring of 2008. Cows were randomly assigned based on age, weight, and days post partum to one of two treatments: 1) Cows with calves separated (CS) synchronized using 5-d CO-Synch + CIDR protocol with calves separated entire 12-h interval between PG injections; 2) Cows with calves returned (CT) and synchronized using 5-d CO-Synch + CIDR protocol with calves returned during 12-h interval between PG injections. CS and CT were processed 72 h following CIDR removal to receive second GnRH and TAI. Variability for AI technicians was accounted for by equally representing them between treatment groups. Bulls were withheld 10 d post TAI and ultrasound pregnancy (US) was determined at 35d via rectal ultrasound with a 2.5 megahertz linear transducer. TAI pregnancy rate for CS was 52.2%, and CT was 55.6% for ranch 1; ranch 2 CS was 67.2% and CT was 72.0%. Ranch one had 172 pregnant and 147 open cows, conception rate of 54%. Ranch two had 75 pregnant and 33 open cows, conception rate of 69%. Based on this study TAI pregnancy rates were not affected by calf separation during the 12-h interval between PG injections using the 5-d CO-Synch + CIDR protocol. Therefore we propose that no adverse or positive effect will occur by leaving the calf separated for the 12-h interval as a means to simplify the application of this TAI protocol.

Key Words: synchronization, beef cows, fixed-time AI

Introduction

There is mounting evidence in the research community that timed-AI pregnancy rates are increased by as much as 10% in beef cows when the duration of CIDR exposure and the interval between GnRH injections is reduced from 7 days to 5 days with the CO-Synch + CIDR protocol. There is also evidence that AI pregnancy rates are improved in beef cows when two injections (compared to a single injection) of prostaglandin (PG) are given 12 hours apart to lyse CLs following CIDR removal (Bridges et al., 2008; Kasimacickam et al. 2009; Kasimacickam et al. 2006). A plausible explanation for the improved response with two injections of PG in the 5-d protocol is that treatment with GnRH at the beginning of the protocol produces ancillary corpora lutea with varied susceptibility to PG during the window following CIDR removal. Therefore two injections of PG appears to increase the number of cows that regress their CL(s) and allow the successful final development and ultimate ovulation of a viable ovarian follicle. When giving two injections of PG in this protocol, the question of the impact of calf separation during the 12-hour interval between injections has arisen. Prior reports with synchronization studies have shown a positive impact of calf removal (Geary et al. 2001, Smith et al. 1976). To-date, this question has not been addressed with the 5-day CO-Synch + CIDR protocol with two PG injections. The primary reason for asking this underlying question in this study pertains more to practicality than to a perceived benefit of 12 h calf removal. Specifically, if there is not a negative impact of removing the calves for the 12-h interval, then it eliminates the need to re-sort the calves from the cows for the second PG injection before they are processed through the chute to avoid injuring the calves. This result would reduce the labor complications "hassle factors" by removing the calves an additional time. Therefore our objective was to determine the effect on fixed-time AI pregnancy rates of separating the calf during the 12-hour interval between the two PG injections following CIDR removal in beef cows synchronized with the 5-day CO-Synch + CIDR protocol.

Materials and Methods

This experiment followed the Colorado State University Animal Care and Use Committee guidelines and regulations. Ranch 1 was located in Akron, CO and had 319 primarily Angus, with some percentage of Limousin and Hereford genetics. Ranch 2, was a producer owned herd in Dublin, Virginia and had 108 crossbred cows. The lactating beef cows were randomized into treatments based on weight, body condition score and days postpartum. Average BCS for ranch 1 was 5.0 and ranch 2 was 5.1. BCS is illustrated below (Figure 1).

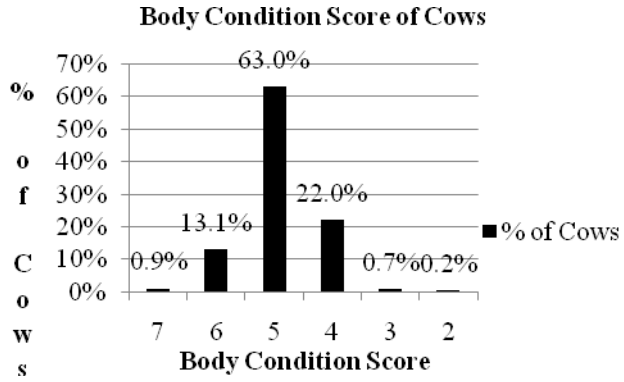

Figure 1: Percent of cows in each Body Condition Score (**BCS**) on both ranches. (BCS was scored on a 1-9 scale; 1=emaciated and 9=obese).

Both groups were synchronized using 5-d CO-Synch + CIDR protocol with a 12-h interval between PG injections (see Figure 1). In TX 1 calves were not returned to their dams following separation to facilitate CIDR removal and were designated as calf separated (**CS**). TX 2 calves were returned after CIDR removal and again separated so that their dams could be processed for the second PG injection. This TX was designated as calves returned (**CT**). Cows in both TX were processed again at 72 h following CIDR removal to receive the second GnRH injection and TAI (Figure 2).

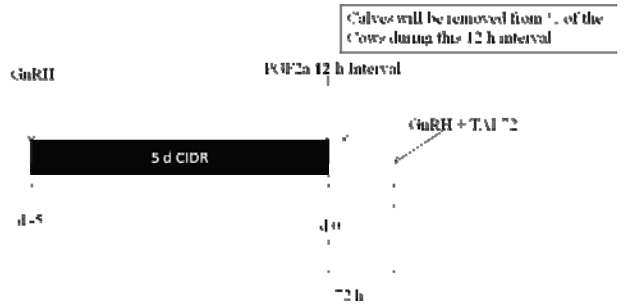

Figure 2: A 5 d CO-Synch +CIDR protocol was used.

Each GnRH dosage was the recommended 2 ml per cow, trade name Cystorelin (Gonadorelin Diacetate Tetrahydrate) marketed by Merial Limited Duluth, Georgia. EAZI-BREED CIDR are marketed by Pfizer Animal Health, New York, New York. CIDR was then left until day 5 and then removed and a PG injection is also given at that time. Each PG dosage was 5 ml per cow IM, trade name Lutalyse, Pfizer Animal Health, New York, New York. Clean up bulls were withheld 10 d post TAI, so that a clear designation between AI and natural service could be established. Pregnancy was diagnosed via trans-rectal ultrasound at day 35 post fixed TAI.

Results of pregnancy diagnosis was tested using PROC FREQ (SAS 9.1), PROC GENMOD (9.1), and PROC GLM adjusting for ranch, TX group, AI technician, body condition score and days post partum. Factors were evaluated based on significant effect at the alpha = .05 level.

Results and Discussion

Eight cows were removed from the study due to a loss of CIDR insert. CIDR's that were lost were recorded at the time of removal and then those cows were removed from the study.

There was not a ranch by TX interaction. TX did not have an effect on pregnancy rate after fixed TAI (P=0.67) which is shown in Table 1.

Table 1: The number of cows in each TX group with respective pregnancy rates.

Treatment (TX) Group	n	Pregnancy Rate per Fixed TAI*
CS (Cows without Calves): 5 d CIDR and 12 h interval between PG injections	215	56.2%
CT (Cows with calves): 5 d CIDR and 12 h interval between PG injections	212	59.4%

*P=0.67

There were also no interactions between technicians, body condition score and days post partum when modeled with pregnancy rates, TX and ranch (no Figure shown). BCS did not have an effect on the pregnancy rates of TX.

Implications

This experiment indicates that there is not a harmful implication of holding the calves away from their dams for 12 h during the PG injection interval on pregnancy rates. Kasimanickam et al., (2009) reported that two PG injections following CIDR removal resulted in 15 to 17% higher TAI pregnancy rate than did a single PG injection with the 5 d CO-Synch + CIDR protocol. It was also reported at the 2008 Roy A. Wallace Memorial Symposium on Bovine Reproduction that several different studies looked at the same PG intervals, and found that they received a higher pregnancy rate as well with a 5 d CO-Synch + CIDR, but that there was no statistical difference between the PG intervals, it was just evident that pregnancy rate increases with two injections vs a single PG injection (Day et al., 2008; Bridges et al. 2008). The current study indicates that there was neither a benefit nor disadvantage in pregnancy rates for leaving the calves separated during the 12 h interval between PG injections. This practice does, however simplify the application of the 5 d CO-Synch + CIRD protocol by eliminating the need to sort the calves from their dams twice during the PG injections.

Acknowledgements

Our research was supported by each ranch's staff and managers; we would like to thank them for the dedicated time they spent working with the cows implementing the protocols. We would also like to acknowledge and thank the following companies for their generous donations of products for the study: Merial Animal Health, Dr. Van Ricketts for the donation of the GnRH, and Pfizer Animal Health, Dr. Dale Grotelueschen for the donation of the CIDRS. This project was funded by the Western Center for Integrated Resource Management at Colorado State University.

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Table 1. Effect of supplemental corn DDGS on performance of steers grazing native range

	DDGS supplementation level, %[a]					Contrast[b]		
Item	0	0.2	0.4	0.6	SE[c]	L	Q	C
Initial BW, kg	203.05	209.35	205.61	204.39	23.6	0.98	0.34	0.47
Final BW, kg	243.03	257.16	256.71	258.05	25.05	.002	.01	0.09
ADG, kg/d	0.71	0.85	0.91	0.96	0.06	0.006	0.31	0.73
Supplement intake, kg/d	0	0.42	0.82	1.24	0.09	<0.01	1.00	0.83
Supplement conversion[d]	-	3.13	6.53	5.37	1.95	0.45	0.38	-

[a]DDGS supplementation was offered at: 0 (no supplement), 0.2, 0.4, and 0.6% of BW.
[b]Probabilities for contrasts: linear (L), quadratic (Q), and cubic (C).
[c]SE with n = 3.
[d]Supplement conversion kg of as-fed DDGS supplement per kg of increased BW gain.

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REPRODUCTIVE PERFORMANCE OF BEEF HEIFERS EXPOSED TO BULLS DURING AN ESTRUS SYNCHRONIZATION PROTOCOL THAT INCLUDED A 14-D CIDR, $PGF_{2\alpha}$, AND, TIMED AI AND GNRH [1]

J. G. Berardinelli, S. A. Tauck, J. R. C. Wilkinson, J. R. Olsen, T. Gibbs, and L. Runnion
Montana State University, Bozeman 59717

ABSTRACT: The objective was to evaluate the estrus synchronization response and AI pregnancy rates of beef heifers exposed to bulls during an estrus synchronization protocol that included a controlled internal drug release devices (CIDR) for 14 d, $PGF_{2\alpha}$ (PG), and, timed AI (TAI) and GnRH. The null hypotheses were that the estrous synchronization response after PG injection and AI pregnancy rates do not differ between heifers exposed or not exposed to mature bulls. Heifers were stratified by birth date, BW, BCS, and presence of a corpus luteum. Heifers were then assigned randomly to be exposed to bulls (BE; n = 41) or not exposed to bulls (NE; n = 41). The heifer to bull ratio was 20.5 to 1. Heifers were exposed to bulls on the day of CIDR insertion (D -32) and remained with bulls until D 3 (D 0 = d of PG injection). CIDRs were removed 14 d (D -18) after insertion. On D 0 each heifer was injected intramuscularly with PG and bulls were removed from BE heifers. Heifers were observed for estrus during the next 60 h from 0600 to 2400 h daily. Heifers that exhibited estrus within 60 h after PG were bred by AI 12 h later. Heifers that did not exhibit estrus by 60 h were TAI at 72 h after PG and given GnRH (100 ug/cow). The proportion of heifers that exhibited estrus after PG was greater ($P < 0.05$) and the interval from PG to estrus was shorter ($P < 0.05$) for NE heifers than for BE heifers. However, there was no difference between intervals to estrus for NE and BE heifers that actually displayed estrus by 60 h after PG. More ($P < 0.05$) NE heifers (65.9%) were bred by AI 12 h after PG than BE heifers (39%), whereas, more ($P < 0.05$) BE heifers were bred TAI at 72 h after PG than NE heifers. Overall AI pregnancy rates for NE and BE heifers did not differ (44.2 and 56.6%, respectively). Although not statistically different, AI pregnancy rates for BE and NE heifers bred 12 h after estrus were 81.3 and 59.3%, respectively ($P = 0.13$). These results indicate that AI pregnancy rates are not altered by exposing heifers to bulls during an estrus synchronization protocol that included CIDR for 14 d, followed 18 d later with $PGF_{2\alpha}$ (PG), and, timed AI (TAI) and GnRH.

Key words: bull, CIDR, estrus synchronization, heifers

Introduction

The occurrence at puberty has a significant effect on reproductive efficiency of beef cattle herds when heifers are bred to calve as 2-yr-olds, particularly in production systems that use restricted breeding seasons (Ferrell, 1982). Additionally, heifers that conceive early in their first breeding season have greater lifetime productivity than do their counterparts that conceive late in their first breeding season (Lesmeister et al., 1973).

Exposing of heifers to oro-nasal treatment of bulls urine (Izard and Vandenberg, 1982) or to bulls while on a high plane of nutrition accelerated the occurrence of puberty. Progestin treatment for 9 or 14 d can induce puberty in beef heifers (Short et al., 1976; Jaeger et al., 1992). Recently, Berardinelli et al. concluded that fixed-time AI pregnancy rates were improved by exposing primiparous, suckled cows to bulls before and during a Hybrid-Synch estrus synchronization (ES) protocol. More importantly, there is the possibility that a pheromone(s) excreted by bulls enhances TAI pregnancy rates in primiparous, suckled cows synchronized with CIDR for 7 d, $PGF_{2\alpha}$, and, timed AI and GnRH. (Tauck et al., 2007).

Based on these observations, we postulated that AI pregnancy rates could be improved by exposing yearling beef heifers to bulls during and ES protocol that included a controlled internal drug release device (CIDR) for 14 d, $PGF_{2\alpha}$, and, timed AI (TAI) and GnRH. The null hypotheses were that the estrous synchronization response after $PGF_{2\alpha}$ administration, and AI pregnancy rates do not differ between heifers exposed or not exposed to mature bulls during an ES protocol that include a CIDR for 14 days.

Materials and Methods

Animals and Treatments. Heifers were housed at the Montana State University, Bozeman Area Research and Teaching Facility. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Agricultural Animal Care and Use Committee.

Eight-two, spring-born, Angus X Hereford heifers and two mature epididymectomized Angus X Hereford bulls were used in this experiment. Heifers were maintained in a single pasture and had no contact with bulls or their excretory products from the previous breeding season until the start of the experiment (D -32). Before the start of the experiment, ovarian functional status of each heifer was

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rated by two ultrasound examinations of each ovary for the presence or absence of a corpus luteum (cycling status). The first and second ultrasonic examinations were conducted 10 and 2 d, respectively, before the start of the experiment. Means for age, BW, and BCS of heifers were 12.8 ± 0.6 months (± SD), 337.3 ± 26.0 kg, and 4.9 ± 0.2, respectfully, at the start of the treatment.

Two d before the start of the experiment heifers were stratified by age, BW, BCS, and cycling status. Once stratified, heifers were assigned randomly within strata to one of two treatments: exposed to continuously to bulls (EB; n = 41) or not exposed to bulls (NE; n = 41) for 32 d from D -32 to D 0.

Facilities and Bull Exposure. Two lots were used for this experiment, designated north and south by their geographic location. Lots were adjacent to each other and separated by a wooden, fixed fence and an additional barb-wire fence that separated EB and NE Heifers by approximately 15 m. Lots were very similar in east-west configuration, bunk space, aspect, and slope.

The heifer to bull ratio was 20.5:1 throughout the exposure period. Heifers in one pen were prevented from seeing or direct, close contact with bulls by tarpaulins draped over the wooden fence.

Nutrition. Heifers were given 12.7 kg•hd $^{-1}$•d $^{-1}$ of good quality, chopped mixed-grass alfalfa hay, 1.1 kg•hd $^{-1}$•d $^{-1}$ cracked barley, 0.45 kg•hd $^{-1}$•d $^{-1}$ supplement that contained 38% protein and 200 mg of Rumensin, water, and a trace mineral-salt supplement throughout the experiment. Heifers were fed one half of the ration in the morning (0800-1000 h) and one half late in the afternoon (1600-1700 h).

Estrus Synchronization, AI, and Pregnancy Diagnosis. Each heifer received a progesterone-containing CIDR 32 d before administration of $PGF_{2\alpha}$ (D 0). Fourteen d later (D -18) CIDRs were removed from heifers and each heifer received $PGF_{2\alpha}$ (25 mg/heifer; i.m.). Each heifer was fitted with an estrus detection aid (Estrotect®; Rockway, Inc., Spring Valley, WI) and observed visually for signs of behavioral estrus. Heifers that showed behavioral estrus and heifers whose estrus-detection aid turned color from silver to red within 60 h after $PGF_{2\alpha}$ were inseminated artificially 12 h later (AI 12). Heifers that did not show estrus and heifers whose estrus-detection aid did not change color within 60 h received GnRH (100 ug/cow; i.m.) and were fixed-time AI (TAI) 72 h after $PGF_{2\alpha}$ (D 3). Heifers that did not exhibit estrus by 60 h after $PGF_{2\alpha}$ were assigned an interval to estrus of 72 h. Semen from 3 bulls of proven fertility were assigned randomly within treatment for insemination of heifers by a single AI technician. Pregnancy rates to AI were determined by transrectal ultrasonography of the uterine contents of each cow 35 d after TAI.

Statistical Analyses. Interval from $PGF_{2\alpha}$ to estrus was analyzed by ANOVA for a completely randomized design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment. Means were separated by the PDIFF procedure of SAS. Proportions of heifers that exhibited luteal structures at the start of the experiment, proportions that showed estrus by 60 h after $PGF_{2\alpha}$; and, AI pregnancy rates for heifers bred at 12 h after estrus, TAI, and overall were analyzed by separate chi-square analyses using the PROC FREQ procedure of SAS.

AI pregnancy rates for semen from the 3 bulls used for heifers in each treatment were analyzed by chi-square analysis using the PROC FREQ procedure of SAS.

Results

Proportions of heifers that had evidence of luteal structures at the start of bull exposure did not differ between treatments; 87.8 and 85.4% for EB and NE heifers, respectively.

Approximately 27% more ($P < 0.05$) NE heifers exhibited estrus or had Estrotects that changed color by 60 h after $PGF_{2\alpha}$ than EB heifers (Table 1). Interval to estrus after $PGF_{2\alpha}$ was longer ($P < 0.05$) for EB heifers than for NE heifers (Table 1). However, there was no difference between intervals to estrus for NE and BE heifers that actually displayed estrus by 60 h after $PGF_{2\alpha}$; 49.5 and 48.8 h, respectively.

More ($P < 0.05$) NE heifers were bred by AI 12 h after $PGF_{2\alpha}$ than BE heifers, conversely, more ($P < 0.05$) BE heifers were bred by TAI at 72 h after $PGF_{2\alpha}$ than NE heifers (Table 1)..

AI pregnancy rates for heifers inseminated 12 h after estrus, TAI pregnancy rates, and overall AI pregnancy rates did not differ between EB and NE heifers (Table 1). Although not statistically different, AI pregnancy rates for BE and NE heifers bred 12 h after estrus were 81.3 and 59.3%, respectively ($P = 0.13$).

AI pregnancy rates for EB and NE heifers inseminated with semen from the 3 bulls did not differ.

Discussion

This study was designed to test whether or not AI pregnancy rates could be improved by exposing yearling beef heifers to bulls during an ES protocol that included controlled internal drug release devices (CIDR) for 14 d, $PGF_{2\alpha}$, and, timed AI (TAI) and GnRH. This hypothesis was based on the findings of Tauck et al. (2007) and Berardinelli and Tauck (2007) that support the concept of a biostimulatory effect of bulls to enhance breeding performance of cows synchronized for estrus using protocols that increase progesterone concentrations for 7 days before administration of a luteolytic agent.

Evaluation of the estrus synchronization response indicated that only 39% of EB heifers exhibited estrus within 60 h after $PGF_{2\alpha}$; whereas, 66% of NE heifers displayed estrus within this period. This result suggests that exposing heifers in combination with 14 d of exposure to progesterone and to bulls for 32 d had a negative effect on their capacity to express estrus $PGF_{2\alpha}$. One explanation for this result may be that heifers exposed to bulls had not ovulated after removal of CIDRs and became anovular. However, 87.8% of heifers assigned to the EB treatment had evidence of luteal tissue in their ovaries at the start of the experiment. So this explanation is not accurate, especially

in light of the observation that AI pregnancy rates did not between EB and NE heifers. Another possibility may be that the interval from $PGF_{2\alpha}$ used for detection of estrus was not long enough for EB heifers to express estrus. It would appear that estrus was delayed, in some manner, in most of the EB heifers (61%). The cause of this delay is not known but is analogous to the observation that estrus was delayed in postpartum, suckled cows that were treated with CIDRs for 14 (see, Tauck et al., 2009 in this Proceedings). Apparently, this delay in exhibition of estrus was not detrimental to TAI pregnancy rate since TAI pregnancy rate of EB heifers did not differ from that of NE heifers.

Although AI pregnancy rates for EB and NE heifers bred 12 h after estrus did not differ significantly ($P = 0.13$), it is interesting to note, that AI pregnancy rate for those EB heifers bred 12 h after estrus was quite high (81.3%; n = 16) relative to that of NE heifers (50.2%; n = 27).

In conclusion, the results of this do not support the hypothesis that AI pregnancy rates can be improved by exposing yearling beef heifers to bulls during an ES protocol that included a controlled internal drug release devices (CIDR) for 14 d, $PGF_{2\alpha}$, and, timed AI (TAI) and GnRH.

Implications

It would appear that combining bull exposure with a estrus synchronization protocol that include controlled internal drug release devices (CIDR) for 14 d, followed 18 d later by $PGF_{2\alpha}$ (PG), and, timed AI (TAI) and GnRH would be beneficial for improving reproductive performance of yearling beef heifers. However, additional research is necessary to evaluate the delay in the estrus synchronization response of heifers exposed to bulls and whether bull exposure may improve AI pregnancy rates in heifers bred 12 h after estrus in this ES protocol.

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Table 1. Percentages of heifers that exhibited estrus by 60 h after $PGF_{2\alpha}$ and interval to estrus after $PGF_{2\alpha}$; percentages of heifers inseminated by AI 12 h after estrus and pregnancy rates for heifers bred AI 12 h after estrus; percentages of heifers timed AI at 72 h (TAI) and TAI pregnancy rates of TAI heifers; and, overall AI pregnancy rates exposed (EB) and not exposed to bulls (NE) during an estrus synchronization protocol that included a 14-d CIDR, followed 18-d later by $PGF_{2\alpha}$, and timed AI and GnRH

	Treatment				
Variable	EB	NE	SEM	X^2	*P* value
n	41	41			
% of heifers that exhibited estrus by 60 h after $PGF_{2\alpha}$	39.0	65.9		5.9	< 0.05
Interval to estrus after $PGF_{2\alpha}$, h	63.3	56.9	12.5		< 0.05
% AI 12 h after estrus	39.0	65.9		5.9	< 0.05
AI pregnancy rates 12 h after estrus[1]	81.3	59.3		2.2	0.13
% TAI 72 h after $PGF_{2\alpha}$	61.0	34.1		5.7	< 0.05
TAI pregnancy rates[1]	32.0	28.6		0.5	0.82
Overall AI pregnancy rates[1]	51.2	48.8		0.5	0.83

[1] Pregnancy rates determined by transrectal ultrasonography of uterine contents 35 d after TAI.

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EFFECT OF 6-H VS 12-H INTERVAL BETWEEN TWO PROSTAGLANDIN INJECTIONS IN BEEF COWS SYNCHRONIZED FOR OVULATION USING THE 5-D CO-SYNCH + CIDR PROTOCOL

Priest, M.K., R.K. Peel, J.C. Whittier, R.M. Enns and G.E.Seidel, Jr.

Colorado State University, Fort Collins

ABSTRACT: Our objective was to determine the effect on fixed-time artificial insemination (**TAI**) pregnancy rates of 6 vs. 12-h intervals between 2 prostaglandin(**PG**) injections following CIDR removal in beef cows synchronized with the 5-d CO-Synch + CIDR protocol. The purpose of this study was to determine if there is flexibility in the time for giving the second PG injection using this protocol. This experiment was conducted on two ranches during the spring 2008. Ranch 1 had 260 mixed commercial & seedstock Angus cows & ranch 2 had 390 seedstock Angus cows. Cows were randomly assigned based on age, weight, and days post partum to 1 of 3 treatments (**TX**): 1) Control (**CNTL**) synchronized using the 7-d CO-Synch + CIDR protocol, utilizing 1 PG injection following removal of the CIDR; 2) 12 h interval between PG injections (**12HI**) synchronized using 5 d CO-Synch + CIDR protocol; 3) 6 h interval between PG injections (**6HI**) synchronized using 5 d CO-Synch + CIDR protocol. All cows were given first GnRH injection when CIDR was inserted. CNTL cows were processed again 60 h post CIDR removal for the second GnRH injection and TAI. 12HI and 6HI were processed 72 h following CIDR removal to receive 2nd GnRH & TAI. All calves were separated from cows before processing and returned following the second PG injection. Variability for AI Technicians was accounted for by random distribution at processing. Bulls were withheld 10 d post TAI so that TAI pregnancy could be determined at 35d via rectal ultrasound. There were no differences (**P<0.05**) in TAI pregnancy rates across all TX groups. Ranch 1 CNTL had a 44.8%, n=87; 12HI had a 50.6%, n=85; & 6HI had a 56.8%, n=88. Ranch 2 CNTL had a 44.2%, n=120; 12HI had a 47.8%, n=136; & 6HI had a 44.0%, n=134. Based on this study pregnancy rates were not affected by 6 h vs. 12 h PG interval injections when the 5 d protocol was used. Also, there was no difference between TAI pregnancy rates when comparing the 7-d and the 5-d protocols, which differ from other recent studies.

Key Words: synchronization, beef cows, fixed-time AI

Introduction

Synchronization methods have been developed in order to facilitate the use of artificial insemination. Recent research and development has focused on methods which will improve the efficacy of timed artificial insemination (**TAI**). Outcomes are more cows calving earlier in the calving season which produce heavier calves, longer postpartum periods for rebreeding, reduced labor for calving season, and opportunity to utilize high accuracy sires (Odde 1990, Johnson 2005, Beal 1998). "CO-Synch" synchronization protocols and the use of an intravaginal progesterone-releasing insert (**CIDR**) are the most widely used programs for TAI in beef cattle (Kasimacickam R, et al. 2009). Bridges et al. (2008) reported that TAI pregnancy rates were increased with the modified 5 d CO-Synch + CIDR program and when GnRH was given 72 h post-PG. Bridges et al. (2008) also found that the overall 5 d program increased 10.5% in pregnancy rates over the 7 d CO-Synch program. The objective of the current study was to determine if there is latitude in the length of the interval between two PG injections with the 5-d CO-Synch + CIDR protocol. This was done by comparing the effect of fixed TAI pregnancy rates of 6 h vs. 12 h intervals between the two PG injections following CIDR removal. Our hypothesis was that the 5 d CIDR protocol would result in higher pregnancy rates. A plausible explanation for the improved response with two injections of PG in the 5-d protocol is that treatment with GnRH at the beginning of the protocol produces ancillary corpora lutea with varied susceptibility to PG during the window following CIDR removal. Therefore two injections of PG appears to increase the number of cows that regress their CL(s) and allow the successful final development and ultimate ovulation of a viable ovarian follicle. The result is that the PG interval would have a similar effect on pregnancy rates when compared. Additionally, our hypothesis included the comparison of both the 5-d CO-Synch + CIDR groups and the 7-d CO-Synch + CIDR groups.

Materials and Methods

This experiment was followed Colorado State University Animal Care and Use Committee guidelines and regulations. Two ranches with crossbred and Angus based beef cows were utilized for this study. Ranch 1, a producer owned herd, had 260 mixed commercial and seedstock Angus cows and ranch 2, a university herd in Saratoga, Wyoming had 390 seedstock Angus cows. The lactating beef cows were randomized based on weight, body condition score and days postpartum into one of three treatment (**TX**) groups. Treatment 1 was the control group (**CNTL**) which was synchronized using the 7-d CO-Synch + CIDR protocol (Figure 1).

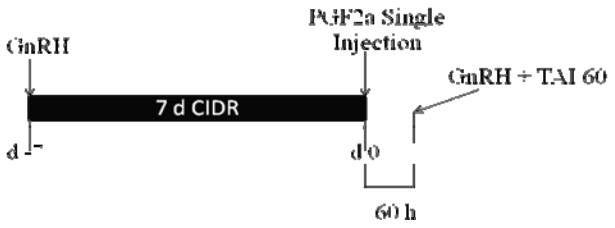

Figure 1: A 7 d CO-Synch + CIDR control group (CNTL).

Treatment 2 was the 12 h interval of 2 PG injections (**12HI**) synchronized using 5 d CO-Synch + CIDR protocol (Figure 2). Treatment 3 was the 6 h interval of 2 PG injections (**6HI**) synchronized using 5 d CO-Synch + CIDR protocol (Figure 2).

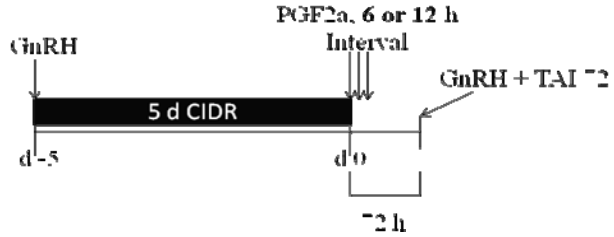

Figure 2: A 5d CIDR + CO-Synch combined with either a 6-h interval (6HI) or a 12-h interval (12HI) between prostaglandin injections.

Calves were sorted from cows before the cows were passed through the chute. Calves were returned when the cows had received their second PG injection. On day -7 or -5 the first GnRH injection was given and CIDR inserted. Each GnRH dosage was the recommended 2 ml per cow, trade name Cystorelin (Gonadorelin Diacetate Tetrahydrate) marketed by Merial Limited, Duluth, Georgia. EAZI-BREED CIDR was marketed by Pfizer Animal Health, New York, New York. CIDR was then left until day 7 or 5 and then removed and the first PG injection given. Each PG dosage was 5 ml per cow IM, trade name Lutalyse, Pfizer Animal Health, New York, New York. The 5 d CO-Synch protocol cows waited the assigned interval time period until the next PG injection which was either 6 h or 12 h after the first PG injection. Control cows were processed again 60 h post CIDR removal for the second GnRH injection and fixed TAI. The 5 d TX groups 12HI and 6HI were processed 72 h following CIDR removal to receive second GnRH and fixed TAI. Clean up bulls were withheld 10 d post TAI, so that a clear designation between AI and natural service conception could be made. Pregnancy was diagnosed via trans-rectal ultrasound at day 35 post fixed TAI.

Pregnancy diagnosis was examined using PROC FREQ (SAS 9.1), PROC GENMOD (9.1), and PROC GLM adjusting for ranch, TX group, AI technician, body condition score and days post partum. Factors were evaluated based on significant effect at the alpha = .05 level.

Results and Discussion

Twelve cows were removed from the study due to a combination of inaccurate records or loss of CIDR insert. Inaccurate records were determined by key pieces of missing information. CIDR's that were lost were recorded at the time of removal and those cows were removed from the study.

Ranch had a significant effect (P=0.04) in the model. There was no ranch by TX interactions. However, TX group pregnancy rate to fixed TAI was not different (P=0.56, Table 1). This result was in contrast to other reported studies which compared 7 d vs. 5 d CO-Synch + CIDR protocols. Bridges et al. (2008) reported that the 5 d CO-Synch + CIDR program increased pregnancy rates across different groups and experiments when compared to the 7 d CO-Synch + CIDR protocol. Kasimanickam et al. (2006) studied the timing of prostaglandin interval injections along with the use of a CIDR and GnRH injections. They reported no statistical differences between the interval periods that affected the TAI pregnancy rate (Kasimanickam et al., 2006).

Table 1: Number of cows and respective TAI pregnancy rates.

Treatment (TX) Group	n	Pregnancy Rate per Fixed TAI
Control (**CNTL**) 7 d CIDR	207	44.4%
12HI: 5 d CIDR and 12 h interval between PG injections	221	48.9%
6HI: 5 d CIDR and 6 h interval between PG injections	222	49.1%

There were no interactions between AI technicians, body condition score and days post partum when modeled with pregnancy rates, TX groups and ranch. Average BCS for ranch 1 was 4.8 and ranch 2 was 4.6. BCS is illustrated below (Figure 3).

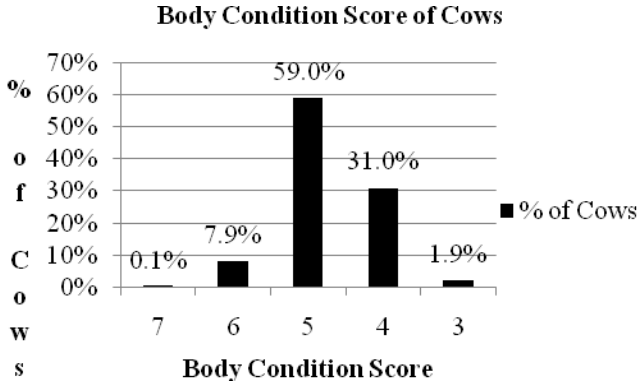

Figure 3: Body Condition Score (**BCS**) of the cows on both ranches. (BCS was scored on a 1-9 scale; 1=emaciated and 9=obese).

Implications

This study shows no significant difference in the PG interval of 6 vs. 12 h and again no difference between the 5 d CO-Synch + CIDR protocol and the 7 d CO-Synch + CIDR protocol. This is in contrast to Kasimanickam et al., (2009) who reported two PG injections resulted in 15 to 17% higher TAI pregnancy rates than single PG injection with the 5 d CO-Synch + CIDR protocol. It was also reported at the 2008 Roy A. Wallace Memorial Symposium on Bovine Reproduction that a higher pregnancy rate was attained using the 5 d CO-Synch + CIDR compared to the 7-d protocol (Bridges et al. 2008, Day et al. 2008). In addition there were no statistical differences between the PG intervals using the 5

d protocol (Day et al., 2008; Bridges et al. 2008). In contrast, our current study did not show difference in TAI pregnancy rates with 5 d vs. 7 d CO-Synch + CIDR protocols.

Acknowledgements

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USE OF 7 OR 14 D CIDR TREATMENTS IN AN ESTRUS SYNCHRONIZATION PROTOCOL THAT INCLUDED $PGF_{2\alpha}$, AND TIMED AI AND GnRH IN POSTPARTUM, SUCKLED BEEF COWS[1]

S. A. Tauck, J. R. C. Wilkinson, J. R. Olsen, P. Olind, K. C. Davis, and J. G. Berardinelli
Montana State University, Bozeman

ABSTRACT: The objective was to compare the estrus synchronization response and AI pregnancy rates of primiparous, suckled beef cows using protocols that included controlled internal drug release devices (CIDR) for 7 or 14 d, $PGF_{2\alpha}$ (PG), timed AI (TAI) and GnRH. The null hypotheses that estrus synchronization response after PG and AI pregnancy rates do not differ between cows synchronized with a CIDR for 7 or 14 d. Data were collected in two trials conducted in 2006 and 2008. Cows were assigned randomly to receive a CIDR for 7 (CIDR7; n = 104) or 14 d (CIDR14; n = 100). Each CIDR14 cow received CIDR on D-31 (D0 = d of PG); CIDR were removed 14 d later (D -17). Cows in CIDR14 treatment were 61d (SD; ± 13d) after calving. CIDR7 cows received CIDR on D -7, 25 d after CIDR14 cows received CIDR. CIDR7 cows received CIDR 86d (SE; ± 13d) after calving. CIDR were removed from CIDR7 cows on D0 and CIDR14 and CIDR7 cows received i.m. PG injection. Cows that were observed exhibiting estrus within 60 h after PG were bred by AI 12 h later, cows that did not exhibit estrus were TAI at 72 h after PG and given GnRH. Proportion of cows that exhibited estrus after PG was greater ($P < 0.01$) and interval to estrus was shorter ($P < 0.01$) for CIDR7 cows than CIDR14 cows. More ($P < 0.01$) CIDR7 cows (70.2%) were bred by AI 12 h after PG than CIDR14 cows (24.0%), whereas, more ($P<0.01$) CIDR14 cows were bred TAI at 72 h after PG than CIDR7 cows. Overall AI pregnancy rates, 35 d after TAI, did not differ between CIDR7 (63.5%) and CIDR14 (64.0%) cows. Interestingly, AI pregnancy rates of CIDR14 cows ≥ 8-yr-old were greater ($P < 0.05$) than those of aged cows given a CIDR for 7 d. In conclusion, using a CIDR for 7 d with PG given on the day of CIDR removal results in an improvement of the estrus synchronization response compared to that of a CIDR for 14 d followed 17 d later by PG. However, both CIDR protocols yield similar and acceptable overall AI pregnancy rates when combined with TAI and GnRH in postpartum, suckled, beef cows.

Key words: CIDR, cows, estrus synchronization,

Introduction

Estrus synchronization protocols that include progestins are effective in synchronizing estrus and improving AI pregnancy rates in primiparous and multiparous beef cows (Lucy et al.,2001). One of the most common progestin-based protocols that can be used to synchronize estrus in postpartum cows includes the use of a controlled internal drug release device (CIDR) followed by $PGF_{2\alpha}$ 7 d later, before or at the time of CIDR removal (Larson et al., 2006). Tauck et al. (2007) reported that an estrus synchronization protocol that included a CIDR for 14 d followed by $PGF_{2\alpha}$ 17 d later successfully synchronized estrus and yielded acceptable AI pregnancy rates in yearling beef heifers (Tauck et al., 2007). Thereafter, Tauck et al. (2007) found that using a 7 or 14 d CIDR protocol resulted in similar and acceptable AI pregnancy rates when combined with TAI and GnRH in primiparous, suckled, beef cows. However, these results were obtained with small numbers of cows per treatment. Herein, we report the results of a larger study that included both primiparous and multiparous, suckled, beef cows.

The objectives were to evaluate the estrus synchronization response and AI pregnancy rates of postpartum, suckled beef cows using protocols that included a CIDR for either 7 or 14 d, $PGF_{2\alpha}$, and timed AI (TAI) and GnRH. The null hypotheses were that the estrus synchronization response after $PGF_{2\alpha}$ injection and AI pregnancy rates do not differ between postpartum, suckled beef cows synchronized with an ES protocol that included a CIDR for 7 or 14 days.

Materials and Methods

Animals and Treatments. Cows were housed and maintained at two locations: the Bozeman Area Research and Teaching Facility (BARTF), Bozeman, MT and the Montana Agricultural Experiment Station at Red Bluff, MT (RB). Locations are separated by a distance of 56 km and primiparous cows were maintained at the BARTF while multiparous cows were maintained at RB. Animal care, handling, and protocols used in these experiments were approved by the Montana State University Agricultural Animal Care and Use Committee.

Cows at both locations were maintained in single pastures for the duration of the experiment. Ovaries of each cow were ultrasonically examined for the presence of a corpus luteum (CL) at 10 and 2 d before the start of the experiment. Cows were stratified by calving date, calf BW, cow BW and BCS, luteal status, and dystocia score. Cows

[1]This study was supported by the Montana Agric. Exp. Sta. and is a contributing project to Multistate Research Project, W1112, Reproductive Performance in Domestic Ruminants.

were assigned randomly within strata to one of two estrus synchronization protocols that included a CIDR for 14 d (CIDR14) or a CIDR for 7 d (CIDR7). At the start of the experiment, 31 d before administration of $PGF_{2\alpha}$, primiparous and multiparous cows were 74 ± 18 d (± SD) and 47 ± 9 d postpartum. Average calving dates for primiparous and multiparous cows were February 14 and March 13, respectively.

Estrus Synchronization, AI, and Pregnancy Diagnosis. Thirty-one d (D -31) before before administration of $PGF_{2\alpha}$, (D 0) each cow in the CIDR14 treatment received a progesterone-containing CIDR; 14 d later (D -17) CIDRs were removed from these cows. Each cow in the CIDR7 treatment received a CIDR on D -7. Seven d later CIDRs were removed from CIDR7 cows and each cows in the CIDR14 and CIDR7 treatment received $PGF_{2\alpha}$ (25 mg/cow; i.m.). Each cow was fitted with an estrus detection aid (Estrotect®; Rockway, Inc., Spring Valley, WI) and observed visually for signs of behavioral estrus. Cows that showed behavioral estrus and cows whose estrus-detection aid turned color from silver to red within 60 h after $PGF_{2\alpha}$ were inseminated artificially 12 h later (AI 12). Cows that did not show estrus and cows whose estrus-detection aid did not change color within 60 h were given GnRH (100 ug/cow; i.m.) and were fixed-time AI (TAI) 72 h after $PGF_{2\alpha}$ (D 3).

Cows that did not exhibit estrus by 60 h after $PGF_{2\alpha}$ were assigned an interval to estrus of 72 h. Assigning an interval of 72 h for cows that did not exhibit estrus within 60 h after $PGF_{2\alpha}$ allows for a less biased estimation of the mean interval to estrus because cows that did not exhibit estrus would not be included in the statistical analysis for interval to estrus after $PGF_{2\alpha}$. If many cows from a given treatment are not included, the estimation of the mean would be biased to reflect an artificially low estimation for that treatment mean compared to a treatment in which only a few cows are not included. The assumption of a fixed time for the maximum interval to estrus after $PGF_{2\alpha}$ accounts for and removes this innate bias. Pregnancy rates to AI were determined by transrectal ultrasonography of the uterine contents of each cow 35 d after TAI (D 38).

Statistical Analyses. Interval from $PGF_{2\alpha}$ to estrus was analyzed by ANOVA for a completely randomized design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment, location, and their interaction. Means were separated by the PDIFF procedure of SAS. Proportions of cows that showed estrus by 60 h after $PGF_{2\alpha}$, that were inseminated at 12 h after estrus , that were TAI, and AI pregnancy rates were analyzed by separate chi-square analyses using the PROC FREQ procedure of SAS.

Cows were then classified by age and interval from $PGF_{2\alpha}$ to estrus was analyzed by ANOVA for a completely randomized design using PROC GLM of SAS (SAS Inst. Inc., Cary, NC). The model included treatment, age class, and their interaction. Means were separated by the PDIFF procedure of SAS. Proportions of cows of age classes, treated with CIDR7 and CIDR14, that showed estrus by 60 h after $PGF_{2\alpha}$, and their AI pregnancy rates were analyzed by separate chi-square analyses using the PROC FREQ procedure of SAS.

Results

There was no location or treatment by location interaction for interval to estrus after $PGF_{2\alpha}$. Likewise there were no differences between locations and parity classification (primiparous and multiparous) for proportions cows that were bred by AI 12 h estrus or TAI, or for AI pregnancy rates. Thus, data for location and parity were pooled and re-analyzed.

More ($P < 0.01$) CIDR7 cows exhibited estrus or had Estrotects that changed color by 60 h after $PGF_{2\alpha}$ than CIDR14 cows (Table 1). Interval to estrus after $PGF_{2\alpha}$ was longer ($P < 0.01$) for CIDR14 cows than for CIDR7 cows (Table 1).

More ($P < 0.05$) CIDR7 cows were inseminated 12 h after estrus than CIDR14 cows (Table 1). AI pregnancy rates for cows inseminated 12 h after estrus, TAI pregnancy rates, and overall AI pregnancy rates did not differ between CIDR14 and CIDR7 cows (Table 1). AI pregnancy rates of cows that were greater than 7-yr-old (8, 9, and 10 yr; n = 15/treatment) were higher ($P < 0.05$) for CIDR14-treated cows (86.7%) than for CIDR7-treated cows.

Discussion

Use of estrus synchronization protocols depends on ease of implementation in cattle production systems. Thus, researchers attempt to identify those protocols that limit the number of times cows are handled and the necessity for estrus detection. Nevertheless, any protocol must result in AI pregnancy rates that are acceptable to producers, generally above 60%. Lucy et al. (2001), in a relatively comprehensive study of estrous synchronization protocols, reported that a CIDR used for 7 d combined with $PGF_{2\alpha}$ at CIDR removal usually yields an acceptable estrus synchronization response and AI pregnancy rates (Lucy et al., 2001). However, eliminating detection of estrus through the use of TAI may result in less than desirable AI pregnancy rates (Larson et al., 2006). Preliminary evidence from our laboratory indicated that the use of an estrus synchronization protocol that includes CIDR for 14 d followed by $PGF_{2\alpha}$ 17 d later produces an a highly synchronized estrus response and acceptable AI pregnancy rates in beef heifers. Similarly, in a preliminary study with postpartum, primiparous, suckled cows, we found that AI pregnancy rates did not differ between cows treated with a CIDR for 7 or 14 days (Tauck et al., 2007).

The present study repeated the work of Tauck et al. (2007) using a greater number of animals per treatment and included postpartum, multiparous, suckled cows. Again, the objective was to evaluate the estrus synchronization response of cows using a protocol that included a CIDR for 14 d followed by $PGF_{2\alpha}$ 17 d after CIDR removal relative to that of protocol that included a 7-d CIDR. In the present study, we found that approximately 46 % fewer cows

treated with a CIDR for 14 d exhibited estrus by 60 h after $PGF_{2\alpha}$ than cows treated with a CIDR for 7 days. This translated into more CIDR7-treated cows being bred 12 h after estrus than CIDR14-treated cows. On the other hand, more CIDR14-treated cows were bred by TAI, 72 h after $PGF_{2\alpha}$ administration. Nevertheless, overall AI pregnancy rates did not differ between CIDR7- and CIDR14-treated cows. In general, AI pregnancy rates for cows bred at TAI are somewhat lower or more variable for than those for cows bred inseminated artificially 12 h after estrus (Lucy et al., 2001; Larson et al., 2006). The reason for the disparity in the estrus response and the similarity of overall AI pregnancy rates between CIDR7- and CIDR14-treated cows is difficult to explain. One possibility may be that CIDR14 cows simply did not respond to this estrus synchronization protocol. It could be that a CIDR for 14 d and the delay of 17 d before $PGF_{2\alpha}$ induced an inhibition of the normal physiological mechanism that signal resumption of ovulatory activity, i.e., this treatment prolonged postpartum anestrus. This explanation seems untenable because overall AI pregnancy rates did not differ between CIDR14 and CIDR7 cows and were quite acceptable (~64%). One would expect that if CIDR14-treated cows did not respond then AI pregnancy rate for these cows would have been drastically lower. A more plausible explanation may be that treating cows with the CIDR14 protocol delayed the estrus synchronization response in some manner. In other words, if we had increased the 60-h observation period for detecting estrus to greater than 72 h then perhaps a majority of the CIDR14-treated cows would have displayed estrus. Nevertheless, a physiological explanation for these observations is not clear but it must reside in mechanisms associated with changes in follicular dynamics of postpartum, suckled cows treated with a CIDR for 14 days.

An interesting observation in the present study was that AI pregnancy rate for CIDR14-treated cows that were 8-yr-old or older (~87%) was greater than that for CIDR7-treated cows that were 8-yr-old or older (~53%). Thus, it would appear that a 14-d CIDR protocol would enhance the reproductive performance of older cows when using AI. Again, a physiologically meaningful explanation for this observation is not clear.

In conclusion, using a CIDR for 14 d with $PGF_{2\alpha}$ given 17 d after CIDR removal delayed the estrus synchronization response compared to that of a CIDR for 7 and $PGF_{2\alpha}$ on the d of CIDR removal. However, both CIDR protocols yield similar and acceptable overall AI pregnancy rates when combined with TAI and GnRH in postpartum, suckled, beef cows.

Implications

From a practical standpoint, the results of this study indicate that the requirement for estrus detection may be eliminated by using an estrus synchronization protocol that includes a CIDR for 14 d followed by $PGF_{2\alpha}$ 17-d later, and fixed-time AI and GnRH in postpartum, primiparous and multiparous, suckled beef cows. AI pregnancy rates associated with this protocol are similar to that of the standard 7-d CIDR protocol. Additional research is necessary to determine physiological mechanisms for the delay in the estrus synchronization response and the enhancement of AI pregnancy rate for CIDR14-treated 8-yr-old or older cows.

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Table 1. Percentages of cows that exhibited estrus by 60 h after $PGF_{2\alpha}$, interval to estrus after $PGF_{2\alpha}$, percentages of cows inseminated by AI 12 h after estrus and pregnancy rates for cows bred AI 12 h after estrus, percentages of cows timed AI at 72 h (TAI) and TAI pregnancy rates of TAI cows, and overall AI pregnancy rates using estrus synchronization protocols that included 14 d (CIDR14) or 7 d (CIDR7) of CIDR

	Treatment				
Variable	CIDR14	CIDR7	SEM	X^2	*P* value
n	100	104			
% of cows that exhibited estrus by 60 h after $PGF_{2\alpha}$	24.0	70.2		43.6	< 0.01
Interval to estrus after $PGF_{2\alpha}$, h	70.4	57.5	10.5		< 0.01
% AI 12 h after estrus	24.0	70.2		43.6	< 0.01
AI pregnancy rates 12 h after estrus[1]	62.5	67.1		0.17	0.68
% TAI 72 h after $PGF_{2\alpha}$	76.0	29.8		43.6	< 0.01
TAI pregnancy rates[1]	65.8	54.8		1.13	0.29
Overall AI pregnancy rates[1]	64.0	63.5		0.06	0.94

[1] Pregnancy rates determined by transrectal ultrasonography of uterine contents 35 d after TAI.

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COMPARISON OF A MODIFIED 5-DAY CO-SYNCH PLUS CIDR PROTOCOL WITH CO-SYNCH PLUS CIDR IN MATURE BEEF COWS

S. K. Johnson[1], J. R. Jaeger[2], K. R. Harmoney[2], and J. W. Bolte[2]

[1]Northwest Research-Extension Center, Colby; KS; [2]Agricultural Research Center, Hays, KS; Kansas State University, Manhattan, KS.

ABSTRACT: The objective of this study was to compare pregnancy rate to fixed-time AI between cows synchronized with a traditional 7-day CO-Synch plus controlled internal drug release (CIDR) protocol to a 5-day CO-Synch plus CIDR protocol when the interval between doses of $PGF_2\alpha$ in the 5-day protocol was 3 h. Postpartum, lactating Angus cross cows (n=177) were stratified by previous treatment, age and calving date, and assigned randomly to one of two treatments. Cows in the control group (7dCIDR; n=88) received GnRH (100 µg i.m.) and CIDR insertion on d -7, CIDR removal and $PGF_2\alpha$ (500 µg cloprostenol i.m.) on d 0 and fixed-timed AI and GnRH at 56 h after $PGF_{2\alpha}$. Treated cows (5dCIDR; n=89) received GnRH and CIDR on d -5, CIDR removal and 2 injections of $PGF_{2\alpha}$ 3 h apart on d 0 and fixed-timed AI concurrent with GnRH at 72 h after the first $PGF_{2\alpha}$. Serum samples were collected 9 or 7 d before and at the start of treatment for 5dCIDR and 7dCIDR, respectively, for determination of concentrations of progesterone. Bulls were introduced 10 d after fixed-timed AI for the remaining 35 d of the 45 d breeding season. Pregnancy rate to AI was determined 31 d after fixed-time AI with transrectal ultrasonography. On d 0, cows were 71 ± 18 d postpartum, 5.9 ± 2.7 yr of age and body condition score 5.1 ± 0.7. Prior to the onset of treatments, a greater ($P < 0.05$) proportion of multiparous (60%) than primiparous (42%) cows were cycling. There were more ($P < 0.05$) cows cycling prior to treatment in the 5dCIDR (62/89; 70%) group than in the 7dCIDR (38/88; 43%) group. Pregnancy rate to fixed-timed AI did not differ with treatment (53.4% and 54.5%; 7dCIDR and 5dCIDR, respectively). Within the conditions of this study, reproductive performance of cows exposed to a 5-d CO-Synch plus CIDR protocol and a 3 h interval between $PGF_{2\alpha}$ injections was similar to cows exposed to a 7-d CO-Synch plus CIDR protocol.

KEY WORDS: Ovulation, CIDR, Fixed-time AI, cows

Introduction

A CO-Synch + CIDR (controlled internal drug release) fixed-timed artificial insemination (AI) protocol is commonly used in lactating beef cows and consists of GnRH and a CIDR administered at the start of the treatment and CIDR removal and $PGF_{2\alpha}$ administration 7 d later (Larson et al., 2006). Bridges et al. (2008) developed a shortened 5-d protocol that resulted in improved pregnancy rates to AI compared with the 7-d CO-Synch + CIDR protocol. A drawback to the 5-d system is that CL induced to form only 5 d earlier by GnRH administration do not regress with a single injection of $PGF_2\alpha$, rather two injections of $PGF_{2\alpha}$ 12 h apart are required (Bridges et al., 2008; Kasimanickam et al., 2009). The 12-h injection interval is not convenient in many production situations, but a shorter interval may be more practical and efficacious. An interval of 3 to 4 h between injections of $PGF_{2\alpha}$ has been used to induce luteal regression in sheep (Hawk, 1973) and may work in this situation.

The objective of this study was to compare pregnancy rate to fixed-time AI between cows synchronized with either a traditional 7-d CO-Synch + CIDR protocol or a 5-d CO-Synch + CIDR protocol with a 3-h interval between doses of $PGF_{2\alpha}$.

Materials and Methods

Postpartum, lactating Angus-cross cows (n = 177) were assigned to one of two treatments on the basis of age, prepartum nutritional treatment, and calving date. Cows in the control group (**7dCIDR**; n = 88) received GnRH (100 µg, i.m.; Fertagyl, Intervet-Schering Plough Animal Health, De Soto, KS) and an intravaginal insert containing 1.38 g of progesterone (EAZI-BREED™ CIDR®, Pfizer Animal Health, New York, NY) on d -7, CIDR removal and $PGF_{2\alpha}$ (500 µg, i.m. cloprostenol; Estrumate, Intervet-Schering Plough Animal Health, De Soto, KS) on d 0 and h 0, and fixed-timed AI and GnRH at 56 h after $PGF_{2\alpha}$ (Figure 1; Dobbins et al., 2006).

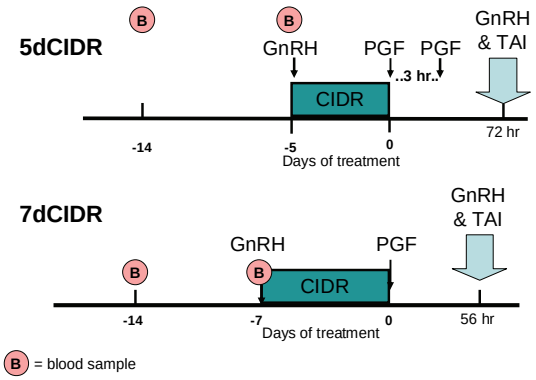

Figure 1. Treatment and sampling schedule. TAI=timed AI.

Treated cows (**5dCIDR**; n = 89) received GnRH and CIDR on d -5, CIDR removal and $PGF_{2\alpha}$ (500 µg cloprostenol) at h 0 and a second injection of $PGF_{2\alpha}$ at 3 h.

Fixed-timed AI was concurrent with GnRH administration at 72 h. Bulls were introduced 10 d after fixed-timed AI. Pregnancy rate to AI was determined 31 d after fixed-time AI with transrectal ultrasonography. Cows grazed native pasture for 1.5 mo prior to the start of the breeding season and remained in one group until d -7. Cows were combined into a single group again following fixed-time AI.

Two serum samples were collected 9 or 7 d before and at the start of treatment for 5dCIDR and 7dCIDR, respectively, for determination of concentrations of progesterone (Figure 1). When one or both samples contained concentrations of progesterone $\geq$ 1 ng/ml cows were considered to be cycling.

Treatment and handling procedures were consistent with normal production practices and were approved by the institutional animal care and use committee.

Binomial data were analyzed using PROC GLIMMIX in SAS (SAS Inst. Inc., Cary NC). The model to test pregnancy rate to AI included fixed effects of treatment, cyclicty, and parity, random effects of sire and technician with body condition score and days postpartum as covariates and appropriate interactions.

Results and Discussion

On d 0, cows were 71 ± 18 d (mean ± SD) postpartum, 5.9 ± 2.7 yr of age and body condition score 5.1 ± 0.7. The mean and distribution of cow body condition, age, and days postpartum is shown in Table 1. Cyclicity differed (P < 0.05) with treatment, parity and days postpartum. A greater proportion of cows 3 years of age or older (60%, n = 146) were cycling prior to the start of treatments compared to 2-year-old cows (42%, n = 31). There were more cows cycling prior to treatment in the 5dCIDR group than in the 7dCIDR group. This difference could be due to the shorter interval between blood samples in the 7dCIDR group resulting in some cycling cows being classified as non-cycling. This compromise on timing of samples was necessary due to labor constraints. Consistent with previous studies (Stevenson et al., 2003), fewer cows were cycling as the interval between calving and breeding was shorter.

Pregnancy rate to fixed-timed AI was 53.4 and 54.5% for 7dCIDR and 5dCIDR, respectively (Table 1). There was no effect of treatment (P = 0.98), cyclicty (P = 0.65), treatment by cyclicity interaction (P = 0.43), parity (P = 0.55), body condition score (P = 0.93) or days postpartum (P = 0.52) on AI pregnancy rate. Numerically, if cows were cycling prior to estrous synchronization, more cows synchronized with the 5dCIDR system conceived to fixed-time AI compared with cows synchronized with the 7dCIDR system (Table 1). In contrast, non-cycling 7dCIDR cows had a numerically higher pregnancy rate to fixed-time AI than 5dCIDR cows. More data is needed to conclude that there is no difference between these treatments. Cows that received the 5-day CIDR protocol and only one injection of $PGF_{2\alpha}$ had 15 to 17% lower pregnancy rates compared to cows that received two injections of $PGF_{2\alpha}$ (Kasimanickam et al., 2009). Final pregnancy rate did not differ between treatments (Table 1).

Some researchers contend that the difference in pregnancy rate between the 7- and 5-d protocols observed by Bridges et al. (2008) can be explained by the 60 h interval to insemination for the 7-d treatment. No difference in pregnancy rate was found between the 7-d and 5-d protocol when fixed-timed AI occurred at 66 h and 72 h, respectively (David Patterson, personal communication) or when both treatments were inseminated at 60 h (Bridges et al., 2008). Interestingly the interval to estrus seemed to differ for theses two studies. Bridges et al. (2008) reported the interval to estrus as 55.9 ± 1.5 h for the 7-d and 58.9 ± 1.3 h for the 5-d treatment, whereas Patterson observed 64.8± 2.6 h (7-day) and 71.2 ± 2.6 h (5-day).

Implications

Pregnancy rate to the 7-d and 5-d CIDR treatments were similar in this study. The 5-d CIDR protocol may be useful in situations where scheduling favors the 5-d over the 7-d system.

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Table 1. Description of cows and pregnancy rates after treatment with either a 7 or 5-d CO-Synch + CIDR protocol[1].

	Treatments	
Item	7dCIDR[1]	5dCIDR[1]
Number	88	89
Cycling, %[2]	43.2	69.7
Non-cycling, %	56.8	30.3
Body condition score, mean	5.1	5.1
Distribution, %		
≤ 4	23.9	34.8
5 to 5.5	61.4	49.4
≥ 6.0	14.8	15.7
Days postpartum[3], mean	72	70
Distribution, %		
> 70 day	52.3	46.1
50 to 70 days	30.7	36.0
< 50 days	17.0	18.0
Years of age, mean	5.8	5.7
Distribution, %		
2	17	18
3-4	17	17
5-9	57	54
> 10	9	11
Pregnancy rates, %		
AI	53.4	54.5
Final	92.0	89.7
Cycling cows (n)	50.0 (38)	57.4 (61)
Non-cycling cows (n)	56.0 (50)	48.1 (27)
Cow age (years)		
2 (n)	73.3 (15)	56.3 (16)
≥ 3 (n)	49.3 (73)	54.2 (72)

[1]Cows received a controlled internal drug-release (CIDR) insert (1.38 g of progesterone) and were administered GnRH (100 µg, i.m.) on d -7 (7dCIDR) or d-5 (5dCIDR). On d 0 and h 0, the CIDR insert was removed and $PGF_{2\alpha}$ (500 µg, i.m. cloprostenol) was administered. At 3 h 5dCIDR cows received $PGF_{2\alpha}$. Fixed-timed AI concurrent with GnRH was at 56 (7dCIDR) or 72 (5dCIDR) h.

[2]Cycling cows had concentrations of progesterone ≥ 1 ng/ml in at least one of two samples collected on d -14 and CIDR insertion.

[3]Days postpartum on the day of $PGF_{2\alpha}$.

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EFFECT OF TARGETED USE OF A CONTROLLED INTERNAL DRUG RELEASING DEVICE (CIDR) IN AN ESTROUS SYNCHRONIZATION SYSTEM FOR POSTPARTUM BEEF COWS

J. B. Hall*, A. Ahmadzadeh, R. H. Stokes, J. K. Ahola, C. Stephenson, J. J. England, and J. B. Glaze, Jr.
University of Idaho, Moscow, ID

ABSTRACT: The objective of this experiment was to determine if BCS, age, and days postpartum could be used as criteria for inclusion of a CIDR in an estrous synchronization program. Crossbred postpartum beef cows (n = 216) were blocked by age and days postpartum and randomly assigned to CO-Synch+CIDR (CO) or modified CO-Synch+CIDR (Target) synchronization system. The CO-Synch+CIDR system consisted of an injection of GnRH (Cystorelin®, 100µg i.m.) and insertion of a controlled internal drug releasing device (Eazi-Breed CIDR®, 1.38 g progesterone) on d 0. On d 7, CIDRs were removed and cows were given PGF2α (Lutalyse®, 25mg i.m.). Cows were bred by fixed-time AI (FTAI) at 60 to 66 h after CIDR removal with GnRH at AI. Cows assigned to the Target system received the same synchronization protocol as CO cows except: 1) cows in BCS ≥ 5, > 3 yr of age, and > 50 d postpartum did not receive a CIDR (n = 53), 2) detection of estrus occurred from CIDR removal until FTAI, 3) cows detected in estrus before FTAI were inseminated 12 h after observed estrus. All cows were fitted with Estrotect estrus detection aids. Pregnancy rates to AI were similar ($P > 0.50$) for CO (60.7%; 65/107) and Target (52.3%; 57/109) cows. There was a tendency ($P = 0.09$) for more cows to be in estrus before FTAI in the CO compared to Target system (62.6% vs. 50.5%, respectively). For cows detected in estrus, AI pregnancy rates were similar ($P > 0.50$) among systems averaging 63.1%. In contrast, for cows not detected in estrus, more CO cows ($P < 0.05$) were pregnant to AI compared to Target cows (60.0% vs. 38.8%, respectively). Strategic use of CIDRs in the Target system reduced synchronization costs by $4.74 per cow. Also, estimated returns per 100 cows favored the Target system by $338. We conclude that targeted use of CIDRs can reduce cost of estrous synchronization without compromising AI pregnancy rate. However, overall return with the Target system should be considered.

Key Words: Postpartum cows, synchronization, cost.

Introduction

The use of estrous synchronization and AI in commercial herds can improve calf marketability and cow productivity. Research trials or case studies reported enhancements in calf weaning weight, the percentage of cows calving in the first 21 days, and returns to cow as a result of estrous synchronization and/AI (Sutphin, 2007). Despite these benefits of estrous synchronization and AI, less than 8% of the US beef cow herds use estrous synchronization or AI (NAHMS, 2009). The principal reasons producers give for not using estrous synchronization or AI are labor and/or time (39.1% and 37.7%, respectively) and cost (16.8% and 21.1%, respectively; NAHMS, 2009).

Labor and time needed for estrous synchronization and AI is reduced by using systems that eliminate estrus detection and inseminate cows at a fixed-time (**FTAI**). The incorporation of a controlled internal drug releasing device (**CIDR**) into estrous synchronization greatly improved synchrony of estrus and pregnancy rates to FTAI (Chenault et al., 2003; Larson et al., 2006) In addition to the increased synchrony, the primary advantage of incorporation of a CIDR in an estrous synchronization protocol is increased estrus response and pregnancy rates in acyclic females (Chenault et al., 2003)

The percentage of postpartum cows cycling before the beginning of the breeding season is affected by age of cow, days postpartum, and body condition score (Short et al., 1990). In a herd, a greater percentage of mature cows (≥ 4 yr) cycle before the breeding season compared with 2 and 3 yr old cows. The percentage of cows cycling before the breeding season increases with increasing d postpartum (Short et al., 1990). Cyclicity is enhanced in postpartum cows with BCS ≥ 5 (1 = emaciated to 9 = obese) compared with cows with BCS < 5 (Richards et al., 1986). Therefore, mature cows in good body condition that calve early in the calving season should be cycling at the beginning of the breeding season.

Many experiments compare the efficacy of estrous synchronization protocols on pregnancy rates to AI; however, few studies compare the relative cost of different protocols (Johnson and Jones, 2005, 2008) as well as their economic impact on cow and calf productivity. Incorporation of economic and calf performance data with reproductive data may increase producer adoption of estrous synchronization systems.

We hypothesized that estrous synchronization costs could be reduced by not using CIDRs in potentially cyclic cows, and that pregnancy rates and/or estimated returns to cow would not be affected by targeted use of CIDRs. The objectives of this experiment were 1) to determine if BCS, age, and d postpartum could be used as criteria for inclusion of a CIDR in an estrous synchronization program, and 2) estimate the economic impact of targeted usage of a CIDR for estrous synchronization and AI.

Materials and Methods

Two hundred and sixteen crossbred postpartum beef cows were blocked by age and d postpartum and

randomly assigned to CO-Synch+CIDR (**CO**) or modified CO-Synch+CIDR (**Target**) synchronization system (Figure 1). The CO-Synch+CIDR system consisted of an injection of GnRH (Cystorelin®, 100µg i.m., Merial, Duluth, GA) and insertion of a controlled internal drug releasing device (Eazi-Breed CIDR®, 1.38 g progesterone, Pfizer, New York, NY) on d 0. On d 7, CIDRs were removed and cows were given PGF2α (Lutalyse®, 25mg i.m., Pfizer). All CO cows were bred by fixed-time AI (FTAI) at 60 to 66 h after CIDR removal with GnRH at AI. Cows assigned to the Target system received the same synchronization protocol as CO cows except: 1) cows in BCS ≥ 5, > 3 yr of age, and > 50 d postpartum did not receive a CIDR (n = 53), 2) detection of estrus occurred from CIDR removal until FTAI, 3) cows detected in estrus before FTAI were inseminated 12 h after observed estrus. Any Target cow not detected in estrus was bred by FTAI at 60 to 66 h after CIDR removal with GnRH at AI.

Cows were inseminated by a single AI technician to 1 of 7 bulls. Bulls were pre-assigned to balance bulls over treatment, cow age, d postpartum, and FTAI d. To ensure cows could be inseminated in a timely manner, half of the cows in each treatment began synchronization treatments 24 h before the remaining cows. Therefore, a maximum of 108 cows could be inseminated at FTAI. Cows were assigned to insemination d to ensure d postpartum, cow age, and bull were not confounded with FTAI d.

Estrus detection was conducted three times daily at 0800, 1300, and 1800 h from injection of $PGF_{2\alpha}$ until the morning of FTAI. To facilitate estrus detection, all cows were fitted with Estrotect® (Estrotect, Inc., Spring Valley, WI) estrus detection aids. Day and time of observed estrus was recorded on all treatments. However, only cows on the Target treatment were inseminated 12h after observed estrus.

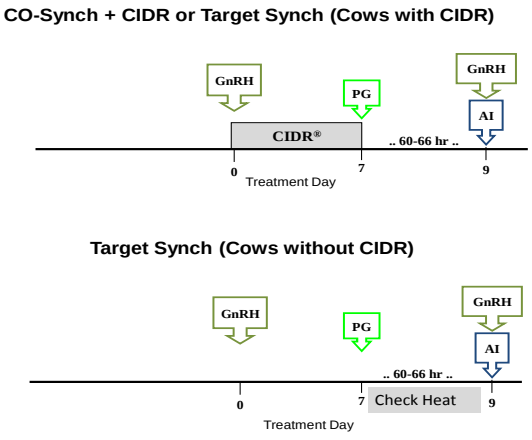

Figure 1. Estrous synchronization protocols for cows receiving CO-Synch+CIDR or Target treatments. Estrus was detected on all cows. Cows on the Target protocol that were observed in estrus were bred AI at 12 h after estrus. At 60 to 66 h after PGF2α, Target cows not observed in estrus and all cows receiving CO-Synch+CIDR were bred by fixed-time AI (FTAI) with GnRH. GnRH = 100µg gonadorelin HCl; PG = 25mg PGF2α (dinoprost); CIDR = 1.38g P4 internal drug releasing device.

Clean-up bulls were introduced 14 d after FTAI, and remained with cows for 45 d. Pregnancy diagnosis was performed via ultrasound on d 55 after FTAI, and final pregnancy diagnosis was via palpation at 90 d after FTAI.

Economic calculations were based on cost and returns per 100 cows with the assumption of a 96% overall pregnancy rate and a 93% weaning rate. Distribution of calves into AI, first natural service and second natural service was based on results from the current project. Prices used for cost of estrous synchronization materials were based on local prices. Cost per dose of CIDR, $PGF_{2\alpha}$, and GnRH were $8.50, $2.50, and $4.25, respectively. The Target treatment had an extra charge of $150 for additional labor. Calf value was based on the prices obtained from calves sold from the University of Idaho NMCREEC in the fall of 2008. Average price for all calves was $113.00/45 kg for a 250 kg calf with a $6.00/45 kg slide. Average weight of calves was set at 267.6 kg, 244.9 kg, and 226.8 kg for AI, first natural service, and second natural service calves, respectively.

The percentage of cows in estrus before FTAI, pregnancy rate to AI, AI pregnancy rate of cows in estrus before FTAI, AI pregnancy rate of cows not observed in estrus, and overall pregnancy rate were analyzed by Chi Square. Economic data were not subjected to statistical analyses as the data were calculated from group means rather than individual calf performance.

Results

Originally 221 cows were used in the project. Five cows were removed from the project for reasons not related to the experiment (i.e. footrot, injury, temperament). The remaining 216 cows were distributed to CO (n = 107) or Target (n = 109) treatment. Target and CO cows were similar ($P > 0.50$) in age (avg. 4.9 ± 0.3 yr), d postpartum (avg. 66.4 ± 1.3d), and BCS (avg. 5.8 ± 0.1). The percentage of cows receiving a CIDR was 100% and 51.4 % for CO and Target cows, respectively.

More cows tended to be detected ($P < 0.09$) in estrus before FTAI in the CO (62.6%) compared with the Target system (50.5%). However, pregnancy rate to AI was similar ($P > 0.5$) among treatments (Figure 2). There was an interaction between synchronization treatment and observed estrus on pregnancy rate to AI. Pregnancy rates were similar ($P > 0.50$) for cows that exhibited estrus regardless of treatment. However, of cows that did not exhibit estrus, FTAI pregnancy rate was lower ($P < 0.05$) in Target vs. CO cows (Figure 3).

Within the Target group, there was no difference ($P > 0.24$) in pregnancy rates for cows receiving a CIDR compared with cows not receiving a CIDR (50.0 vs. 54.7%, respectively). Similarly, there was no effect of a CIDR regardless of estrus response in the Target group.

Strategic use of CIDRs in the Target system reduced synchronization costs by $4.74 per cow (Table 1). Estimated gross calf sales per 100 cows were $140 more for the CO vs. Target system (Table 2). However, estimated returns per 100 cows favored the Target system by $338 over the CO system due to reduced synchronization costs (Table 3).

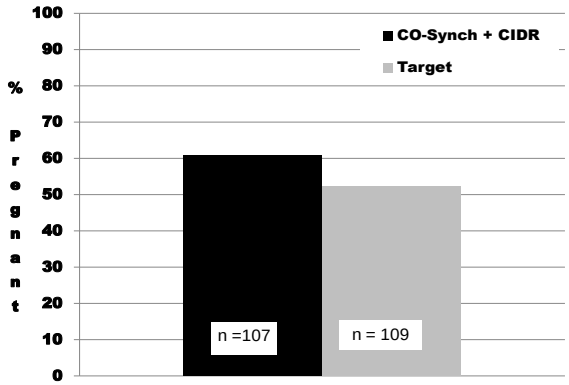

Figure 2. Pregnancy rates to AI for cows synchronized with the CO-Synch+CIDR or Target protocol. ($P > 0.50$)

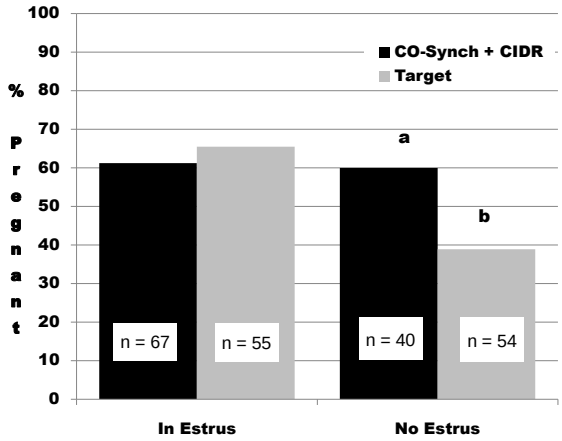

Figure 3. Effect of estrus status before insemination on AI pregnancy rates in cows synchronized with CO-Synch+CIDR or Target protocols. [a,b] Means within estrual status differ ($P < 0.05$).

Discussion

In the present study, targeted use of CIDRs in a CO-Synch+CIDR system did not affect pregnancy rates to AI, but did decrease cost of synchronization and slightly increased returns per 100 cows. This economic advantage was maintained despite increased labor costs.

The percentage of cows in the Target group that did not receive a CIDR, approximately 50%, may be representative of what could be expected in a typical commercial herd. However, the reason for receiving a CIDR may not have represented a typical herd. In this study, a majority of the cows in the Target group that received a CIDR were young cows (47/53). Only one cow received a CIDR due to poor body condition with the remainder of the cows receiving CIDRs because they were < 50 d postpartum. The percentage of young cows in the herd is an artifact of herd building at this research station. A more typical commercial herd would have fewer young cows, but may have more cows that were thin or early postpartum.

Pregnancy rate to AI in the present study was unaffected by synchronization system and averaged in excess of 55%. These results are similar to previous reports of AI pregnancy rates in postpartum cows using CO-Synch + CIDR with FTAI (Chenault et al., 2003; Larson et al., 2006). Pregnancy rates for cows detected in estrus before AI (> 60%) are consistent for FTAI systems and limited estrus detection plus FTAI systems.

In contrast, for cows not detected in estrus, the decreased pregnancy rates in the Target system relative to the CO system may indicate that some cows that did not receive a CIDR in the Target system may still have been anestrus. We did not assess cyclic status via circulating progesterone concentrations which is a limitation of this study. Alternatively, omitting CIDR from some cows may have resulted in decrease synchrony and estrus before PGF2α. Approximately, 5-15% of cows synchronized with the CO-Synch protocol without a CIDR will be in estrus before prostaglandin administration (Chenault et al., 2003). By design, we did not perform estrus detection before prostaglandin injection to limit the amount of additional labor with the Target system with the knowledge that a few cows may not be inseminated at the proper time. However, within the Target group, pregnancy rates were not affected by presence of a CIDR.

Cost and income estimates were based on actual prices incurred or received at the research station during the project. Number of calves per calf type was based on actual pregnancy results from the present experiment. Weights by calf type were estimated from typical weaning weight at the station and the observation that weaning weight decreases approximately 20 to 25 kg for every 21 d decrease in age at weaning. The difference in return to management and labor was minimal with a $3.88/cow advantage for Target cows. Although we adjusted weight for age differences, we did not include a "premium" for the potential increase in genetic value of the calf. When this value can be captured (Sutphin, 2007; Johnson and Jones, 2008), the difference between this system would be negligible or may favor the CO-Synch system.

Implications

Targeted use of CIDRs can reduce costs associated with estrus synchronization without compromising AI pregnancy rates. However, the overall economic benefit may not be as great as perceived by producers or AI technicians when impacts on weaning weight and calf value are taken into account. Comparisons of different estrus synchronization systems should report economic as well as reproductive impacts.

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Table 1. Product cost and costs of additional labor[1] for cows synchronized by CO-Synch+CIDR or Target systems

		Estrous Synchronization Protocol			
		CO-Synch + CIDR		Target	
Product/item	Cost/dose	No. of doses	Cost/100 Hd	No. of doses	Cost/100 Hd
CIDR	$8.50	100	$850.00	51	$433.50
PGF	$2.50	100	$250.00	100	$250.00
GnRH	$4.25	200	$850.00	150	$637.50
Extra labor			0		$150.00
Total Cost			$1,950.00		$1,471.00
Cost per cow			$19.50		$14.71

[1]Labor in addition to 3 cattle workings associated with CO-Synch+ CIDR protocol

Table 2. Impact of synchronization system on estimated calf performance, weaning wt., and total calf weight per 100 cows.

		Estrous Synchronization Protocol			
		CO-Synch + CIDR		Target	
Calf Type	Ave. WW, kg	No. of calves	Total Weight, kg	No. of Calves	Total Weight, kg
AI	267.6	60	16,054	51	13,646
1st Natural Service	244.9	20	4,898	20	4,898
2nd Natural Service	226.8	13	2,948	22	4,989
Total		93	23,900	93	23,533

Table 3. Comparison of estimated income and expenses as affected by synchronization system.

Income			Expenses		
	CO-Synch+CIDR	Target		CO-Synch+CIDR	Target
Avg. calf weight, kg	257.0	253.0	Synchronization cost	$1950.00	$1471.00
Price per 45kg	$112.00	$112.52	Semen cost	$1500.00	$1500.00
Total kg sold	23,900	23,533	AI supplies and estrus detection aids	$300.00	$300.00
Gross income per 100 cows	$59,024.00	58,883.43	Total Cost	$3,750.00	$3,271.00
Difference (CO – Target)	$140.57			$479.00	

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DURATION OF DAILY BULL EXPOSURE ON RESUMPTION OF OVULATORY ACTIVITY IN POSTPARTUM, PRIMIPAROUS, ANOVULAR, SUCKLED, BEEF COWS[1]

S. A. Tauck, J. R. Olsen, J. R. C. Wilkinson, and J. G. Berardinelli
Montana State University, Bozeman 59717

ABSTRACT: The objective of this experiment was to determine if duration of daily bull exposure influences length of postpartum anestrus in primiparous, anestrous, suckled cows. The null hypotheses were that intervals from calving or the start of bull exposure (D 0) to resumption of ovulatory activity (OA) and proportion of cows that resumed OA during the experiment does not differ among cows exposed to bulls 0, 6, and 12-h daily and there is no relationship between the duration of bull exposure and interval to resumption of OA in cows exposed to bulls 0, 6, or 12-h daily. At, 51.5 ± 2.3 d (± SE) cows were assigned randomly to be exposed 12 (BE12; n = 15) or 6 h daily (BE6; n = 14) to bulls or not exposed to bulls (NE; n = 10) for 45 d. Mean interval from calving and D 0 to resumption of OA were shorter ($P < 0.05$) and the proportion of cows that resumed OA during the experiment was greater ($P < 0.05$) for BE12 than NE cows. Interval from D 0 to resumption of OA did not differ ($P > 0.10$) between BE6 cows and either BE12 or NE cows. However, interval from calving to resumption of OA was shorter ($P < 0.05$) for BE6 than NE cows. The proportion of cows that resumed OA did not differ ($P > 0.10$) between BE6 cows and BE12 cows; however, the proportion of cows that resumed OA during the experiment tended ($P = 0.08$) to be greater for BE6 cows than for NE cows. There was a linear relationship between interval from calving ($b_1 = -7.64$ d/h; $P < 0.05$) and D 0 ($b_1 = -3.3$ d/h; $P < 0.05$) to resumption of OA and duration of daily bull exposure. Thus, the duration of bull-pheromone stimuli that cows perceive each day is related to when primiparous, postpartum, anestrous, suckled cows respond to this stimulus and undergo the physiological changes necessary to resume of ovulatory activity.

Key words: **b**iostimulation, pheromone, postpartum anestrus

Introduction

Exposing primiparous and multiparous cows to close physical contact (Custer et al., 1990), fence-line contact (Berardinelli and Tauck, 2007) with bulls accelerates resumption of ovulatory activity (OA) sooner after calving than cows not exposed to bulls. This effect termed the "biostimulatory effect of bulls" is mediated by pheromones released into the environment by the excretory products of bulls (Berardinelli and Joshi, 2005). There is evidence to indicate that duration of daily pheromonal stimuli influences the time required for cows to respond to the biostimulatory effect of bulls. Fernandez et al. (1996) reported that interval from calving to resumption of OA was not altered in cows exposed cows to bulls for 2-h every third day for 18 d beginning 33 d after calving compared to cows exposed continuously to bulls. However, postpartum anestrus was attenuated in cows exposed to the excretory products of bulls for 12-h daily (Berardinelli and Joshi, 2005). These data indicate that the number of days required to accelerate resumption of OA in anestrous cows decreases as cows perceive pheromones produced by bulls for longer or more frequent intervals each day. However, the relationship between duration of daily pheromone exposure and intervals from calving or exposure to resumption of OA it is not known.

The question asked in this experiment was, "Is the interval from calving or the start of bull exposure to resumption of OA related to the duration of daily bull exposure in primiparous, postpartum, anestrous, suckled beef cows?" The objectives of this experiment were to determine if resumption of OA is accelerated in primiparous, anestrous, suckled cows exposed to bulls for 0, 6, or 12 h daily; and if so, are the intervals from calving or the start of bull exposure to resumption of OA related linearly to the duration of daily bull exposure.

Materials and Methods

*Animals and Treatments.*Cows were housed at the Montana State University Bozeman Area Research and Teaching Facility. Animal care, handling, and protocols used in this experiment were approved by the Montana State University Agricultural Animal Care and Use Committee.

Thirty-nine, spring-calving, two-yr-old Angus X Hereford primiparous, suckled beef cows and four mature epididymectomized Angus X Hereford bulls were used in this experiment. Cows and calves were maintained in a single pasture and had no contact with bulls or their excretory products from the previous breeding season until the start of the experiment (D 0). Average calving date for these cows was Feb. 18. Before the start of the experiment, ovarian functional status of each cow was rated by two

[1]This study was supported by Award No. 2007-35203-17743, NRI Competitive Grants Program, CSREES, and the USDA, the Montana NSF EpsCOR program, the Montana Agric. Exp. Sta., and is a contributing project to Multistate Research Project, W1112, Reproductive Performance in Domestic Ruminants.

ultrasound examinations of each ovary for the presence or absence of a corpus luteum. The first and second ultrasonic examinations were conducted 10 and 2 d, respectively, before the start of the experiment. Cows that did not have of a corpus luteum on either ovary in both ultrasound examinations were used in this experiment.

The interval from calving to D 0 averaged 51.5 ± 2.3 d. Two d before the start of the experiment cows were stratified by calving date, calf birth weight, dystocia score, cow body weight, cow BCS, and sex of calf and assigned randomly to be exposed to bulls for 12 h daily (BE12; n = 15), 6 h daily (BE6; n =14) or not exposed to bulls (NE; n = 10) for 45 d.

Facilities and Bull Exposure. Cows were housed within pens in separate lot areas. Pens within the south lot were used to maintain BE12 and BE6 cows while pens within the north lot were used to maintain NE cows. A common holding pen, approximately 0.35 km from the lot that housed NE cows and 30 m from the lot that housed BE12 and BE6 cows, was used to house bulls before and after daily exposure periods. During daily exposure periods two bulls were moved from the common holding pen into the pen that housed BE12 cows and two bulls were moved into the pen that housed BE6 cows. Cows in each treatment were exposed to bulls at 0700 h each day for 45 d (D 0 to 44). At 1900 and1300 h bulls were removed from pens that housed BE12 and BE6 cows, respectively, and housed in the common holding pen until the following day. Cows in each treatment could not see or smell bulls before or after daily exposure periods. Fig. 1 illustrates pen arrangements and method of daily bull exposure

Nutrition. Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available before the start of the experiment. Once cows and calves were moved into pens they were given free access to the same hay, 0.5 kg•hd^{-1}•d^{-1} cracked corn, water, and a trace mineral-salt supplement. Average body weight of cows was 467.5 ± 37.7 kg. The TDN of the diet was 110% of the recommended energy requirement for lactating beef cows with a mature weight of 533 kg (NRC, 1996). Bulls were fed the same diet as cows.

Blood Sampling, Progesterone Concentrations, and Resumption of Ovulatory Activity. Blood samples were collected from each cow by jugular venepuncture every other day from D 0 to the end of the experiment (D 44). Serum was assayed for progesterone concentration in duplicate using solid-phase RIA kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) validated for bovine serum in our laboratory (Custer et al., 1990). Intra- and interassay CV for a serum pool that contained 2.2 ng/mL of progesterone were 10.2 and 15.4%, respectively, and 8.9 and 11.8%, respectively, for a pool that contained 5.75 ng/mL. Progesterone concentrations in these samples were used to determine the interval from calving to resumption of OA, interval from the start of the experiment to resumption of OA, and the proportion of cows that resumed OA during the experiment. An increase of progesterone concentration, above the average progesterone baseline of individual cows in three consecutive samples that exceeded 1 ng/mL was used to determine the occurrence of resumption of OA. Intervals from calving and D 0 to resumption of OA were determined by the number of days from the treatment to the lowest inflection point before a rise in three consecutive samples that exceeded 1 ng/mL. Progesterone concentrations and resumption of OA were confirmed by transrectal ultrasonographic examination of ovaries of each cow using a Titan ultrasound with a 7.5 to 10 MHz rectal transducer every other day throughout the 45-d exposure period used in this experiment (SonoSite Inc., Bothell, WA, USA). The presence of a corpus luteum in the same anatomical position of an ovary in 4 successive scans was used as evidence to confirm resumption of OA. Cows that failed to exhibit a rise in progesterone over three consecutive samples and did not have a corpus luteum in their ovaries were assigned an interval from calving or the start of treatment to the end of the experiment.

Statistical Analyses. Intervals from calving and D 0 to resumption of OA were evaluated by ANOVA for a completely randomized design using PROC GLM of SAS (SAS, Cary, NC). The model included treatment (TRT) and means were separated using Bonferroni Multiple Comparison tests. Linear regression analyses were used to determine the relationship between intensity of bull exposure (hours/d) and intervals (days) from calving and the start of the experiment to resumption of OA using the PROC REG procedure of SAS. Data for intervals from calving and from D 0 to resumption of OA showed heterogeneous variances among treatments using Bartlett's Box *F*-test. Therefore, data from calving and the start of the experiment to resumption of OA that were used in ANOVA were transformed by raising intervals to the power of 6 and 10.3, respectively. Least squares means and standard errors of means for intervals from calving and D 0 to resumption of OA, reported herein, were transformed to original values after analysis. Linear regression analyses were conducted by raising intervals from calving and D 0 to resumption of OA and hours of daily bull exposure to the power of 6 and 10.3, respectively. The sixth and tenth-third root of slopes and Y-intercepts for regression lines for interval from calving and D 0 to resumption of OA, respectively, were used to estimate days for Y-intercepts and d/h for slopes. Differences in proportions of cows that resumed OA during the experiment were analyzed by chi-square using the PROC FREQ procedure of SAS.

Results

Mean interval from calving and D 0 to resumption of OA was shorter ($P < 0.05$) for BE12 cows than for NE cows (Table 1). Additionally, mean interval from calving to resumption of OA for BE6 cows was shorter ($P < 0.05$) than for NE cows, but did not differ ($P > 0.10$) from BE12 cows (Table 1). Interval from D 0 to resumption of OA did not differ ($P > 0.10$) between BE6 cows and either BE12 or NE cows (Table 1). More ($P < 0.05$) BE12 cows resumed OA during the experiment than NE cows (Table 1). The

proportion of cows that resumed OA did not differ ($P > 0.10$) between BE6 cows and BE12 cows; however, the proportion of cows that resumed OA during the experiment tended ($P = 0.08$) to be greater for BE6 cows than for NE cows (Table 1).

There was a linear relationship ($b_1 = -7.64$ d/h; $P < 0.05$) between interval from calving to resumption of OA and hours per day that cows were exposed to bulls (Fig. 2). Likewise, there was a linear relationship ($b_1 = -3.3$ d/h; $P < 0.05$) between interval from D 0 to resumption of OA and hours of daily bull exposure (Fig. 3).

Discussion

In the present experiment, intervals to resumption of OA were shorter and the proportions of cows that resumed OA by the end of the experiment was greater for cows exposed to bulls for 12 h daily than for cows not exposed to bulls. These data are consistent with those of Berardinelli and Joshi (2005) who reported that a greater proportion cows exposed to excretory products of bulls for 12 h daily resumed OA sooner than cows not exposed to bulls or the excretory products of cows. Taken together, these results indicate that exposing postpartum, anestrous cows to presence of bulls or their excretory products for 12 h daily accelerated resumption of OA.

Interestingly, intervals from calving and start of the experiment to resumption of OA did not differ between cows exposed to bulls for 6 h daily and cows exposed to bulls for 12 h daily. However, interval from calving to resumption of OA was shorter for cows exposed to bulls for 6 h daily than for cows not exposed to bulls and the proportion of cows that resumed OA during the experiment tended to be greater for cows exposed to bulls for 6 h daily than for cows not exposed to bulls. These data indicate that the biostimulatory response of cows exposed to bulls for 6 h daily was intermediate between that of cows exposed to bulls for 12 h daily and for cows not exposed to bulls. Therefore, the biostimulatory effect of bulls on resumption of OA appears to rely on duration of daily bull exposure in a dose-dependent manner.

Results of the present experiment indicate that cows responded to pheromonal stimuli from bulls in a dose-dependent manner. Cows that did not perceive the minimum daily dose or threshold of pheromone stimulation and relaxation cycles would not receive the appropriate biostimulatory signal and would not resume OA. However, as duration of daily bull exposure increased, the opportunity for cows to perceive daily pheromone stimulation and relaxation cycles also increased. When postpartum anestrous cows perceived a threshold dose or number of pheromone stimulation and relaxation cycles each day they responded to the biostimulatory effect of bulls by resuming OA. The dose-dependent manner by which pheromones produced by bulls accelerated resumption of OA in postpartum anestrous cows may explain disparate reports in the literature concerning fence-line contact and intermittent exposure of cows to bulls. Shipka and Ellis (1998; 1999) reported that fence-line contact of cows with bulls separated by 6 to 8 m did not accelerate resumption of OA; whereas, Fike et al. (1996) and Berardinelli and Tauck (2007) reported that nose-to-nose fence-line contact of cows with bulls accelerated resumption of OA in postpartum, anestrous cows. Combining the result of the present experiment with those of Shipka and Ellis (1998; 1999), Fike et al. (1996) and Berardinelli and Tauck (2007), one could conclude that cows separated from bulls by 6 to 8 m may not sense sufficient doses of bull-pheromonal stimuli each day to cause a biostimulatory effect. On the other hand, cows in close, nose-to-nose contact with bulls may sense thresholds of bull-pheromonal stimuli each day that activate the pheromonal-biostimulatory pathway and accelerate resumption of OA. The hypothesis that cows sense and respond to pheromones produced by bulls in a dose-dependent manner may explain the results of Fernandez et al. (1996) who reported that cows exposed to bulls for 2 h every 3 d for 18 d did not resume OA sooner than cows not exposed to bulls. Simply put, exposing cows to bulls for 2 h every 3 d for 18 d may not deliver sufficient doses of daily bull-pheromonal stimulus that are required to accelerate resumption of OA in postpartum anestrous cows. Thus, the concept that cows respond to bull-pheromonal stimulation and relaxation cycles in a dose-dependent manner may be a critical component of the pheromonal mechanisms by which the biostimulatory effect of bulls accelerates resumption of OA in primiparous, postpartum, anestrous, suckled cows.

In conclusion, exposing primiparous, postpartum, anestrous, suckled cows to bulls for 12 h daily reduced the intervals from calving and the start of bull exposure to resumption of OA and increased the proportion of cows that resumed OA within 45 d after the start of bull exposure. Furthermore, intervals from calving and the start of exposure to resumption of OA were linearly related in a dose-dependent manner to the duration daily of bull exposure.

Implications

The specific physiological mechanism whereby the pheromonal stimulus of bull accelerates resumption of ovulatory activity of anovular, suckled cows is not well-understood. However, the duration of bull-pheromonal stimuli that cows perceived each day may be an integral component involved with the biostimulatory effect of bulls to accelerate resumption of ovulatory activity in postpartum, anovular, suckled, beef cows.

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Table 1. Number of cows per treatment and least squares means for intervals from calving and from the start of the experiment (D 0) to resumption of ovulatory activity (OA) and proportions of cows that resumed OA for primiparous, anestrous, suckled, beef cows exposed to bulls for 12 h daily (BE12), 6 h daily (BE6) or not exposed to bulls (NE) for 45 d starting 51.5 ± 2.3 d (± SE) after calving

	Treatment[1]				
Variable	BE12	BE6	NE	SEM	*P* value
n	15	14	10		
Interval from calving to resumption of OA, d[2]	85.5[a]	91.3[a,b]	101.9[c]	13.6	<0.05
Interval from D0 to resumption of OA, d	37.4[a]	41.4[a,b]	44.4[b]	7.0	0.06
Proportion that resumed OA during the experiment, %	60.0[a]	42.9[a,b]	10.0[b]	6.23[3]	<0.05

[1]Means and proportions that lack common superscripts differ ($P < 0.05$).
[2]Cows that failed to exhibit a rise in progesterone over three consecutive samples were assigned an interval from calving or from D 0 to the end of the experiment (D 44).
[3]X^2 value.

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GROWTH AND ATTAINMENT OF PUBERTY IN CALVES FROM BEEF COWS SUPPLEMENTED WITH LINSEED MEAL DURING LATE GESTATION

B. R. Ilse[1], V. L. Anderson[1], J. D. Kirsch[2], D. S. Buchanan[2], K. A. Vonnahme[2]
[1] Carrington Research Extension Center, North Dakota State University, Carrington, ND, USA
[2] Department of Animal Sciences, North Dakota State University, Fargo, ND, USA

ABSTRACT: This study examined the effects of supplementing beef cows with phytoestrogen rich linseed meal (LSM) during late gestation on calf growth and reproduction. Multiparous cows (n = 72) were allotted randomly to one of 12 pens, with six pens supplemented with pelleted LSM and six pens fed a control sunflower meal (SFM) pellet. Diets were formulated to be isocaloric and isonitrogenous. Treatment supplements were included in a totally-mixed ration each day for the last 60 d of gestation. Steer calves (n = 41) were followed from birth to weaning (170 d of age). Heifer calves (n = 30) were followed from birth to 266 d of age. Birth wt, actual weaning wt, and ADG were recorded for both steer and heifer calves. On d 182 of age, and every 14 d until 280 d of age, heifers were weighed, and jugular blood samples collected and serum analyzed for progesterone. Steer birth and weaning wt were not different between treatments ($P > 0.19$; 43.99 vs. 44.76 ± 1.42 kg; 254.84 vs. 270.11 ± 7.70 kg, for LSM vs. SFM, respectively). Steer ADG was not different due to treatment ($P = 0.18$; 1.22 vs. 1.28 ± 0.03 kg, for LSM vs. SFM, respectively). Heifer birth and weaning wt were not different between treatments (P > 0.45; 41.86 vs. 41.12 ± 1.53 kg; 240.30 vs. 250.96 ± 9.29 kg, for LSM vs. SFM, respectively). Overall heifer ADG was not different due to supplementation ($P = 0.84$; 0.83 vs. 0.84 ± 0.05 kg, for LSM vs. SFM, respectively). Previous studies indicated that some heifers start to cycle at 7 mo of age. We observed that 9 heifers attained puberty before 6 mo of age (4 SFM and 5 LSM). Age at puberty (181.2 vs. 187.0 ± 7.0 d, for LSM vs. SFM, respectively) was not affected by treatment ($P = 0.58$). Supplementation of LSM during late gestation does not appear to impact growth rate in calves or the onset of puberty in heifer calves.
Key words: phytoestrogen, linseed meal, cattle

Introduction

North Dakota is the national leader in flax production (USDA, NASS 2008). Linseed meal (LSM) is a byproduct of flax where the oil has been removed and is commonly used in livestock diets. Tou et al. (1998) found that 10% flaxseed fed to rat dams during gestation and lactation influenced reproductive parameters in the female offspring such as decreased age to puberty and lengthened estrous cycle. Protein supplementation in beef cattle during the last trimester of pregnancy increased heifer weaning wt, adjusted 205 d wt, pre-breeding wt, weight at pregnancy diagnosis and had a positive effect on pregnancy rates of heifer calves (Martin et al., 2006).

We hypothesized that 10% LSM supplementation of the maternal diet during late gestation would influence calf growth and reproductive development.

Materials and Methods

Animals and Diets

This study was approved by the North Dakota State University Animal Care and Use Committee.
At approximately 215 d of gestation, multiparous, mixed breed cows (n = 72) were randomly assigned to one of two treatments: 1) 10% LSM pelleted supplement or 2) a control supplement, sunflower meal (SFM). Pelleted supplements were offered (2.2 kg per hd/d) in a totally-mixed ration (Table 1) until parturition. Cows were assigned to treatments using cow weight as a blocking criterion. Additionally calf birth weight and previous calf birth weight were equalized between treatments as much as possible. Animals were allotted to 1 of 12 pens, with six pens supplemented with LSM and six pens fed the SFM pellet. Diets were formulated to provide required nutrients for ~ 670 kg late gestation, mature beef cow as suggested by the National Research Council (NRC, 2000). Upon parturition, cows were comingled and cow-calf pairs managed similarly. Calves were weaned at an average age of 170 d. Steer calves (n = 41) were followed from birth to weaning (170 d of age). One steer calf removed from the experiment which was not due to treatment. Heifer calves (n = 30) were followed from birth to 266 d of age. Birth wt, actual weaning wt, and ADG were recorded for both steer and heifer calves. On d 182 of age, and every 14 d until 280 d of age, heifers were weighed, and jugular blood samples collected and serum analyzed for progesterone (P_4). Blood samples were immediately placed on ice and serum was stored at -20°C until assayed for P4. Progesterone concentrations >1 ng/ml indicated that the heifer had reached puberty. Heifers were managed similarly and fed as suggested by the National Research Council (NRC, 2000) throughout the course of the study.

Analysis of plasma and assays

Serum samples were analyzed for P_4 concentrations by competitive chemiluminescent immunoassay (Immulite 1000, Siemens, Los Angeles, CA).

Statistical analysis

Data were analyzed by least squares (Proc Mixed, V.9.1; SAS Inst. Inc., Cary, NC). Pen was the experimental unit for weaning weight, period weights, ADG and progesterone levels. The statistical model included the fixed effects of gestational diet of the cow and cow weight block.

Results and Discussion

Growth performance

Steer birth wt and weaning wt were not affected by treatment (P > 0.19; 43.99 vs. 44.76 ± 1.42 kg; 254.84 vs. 270.11 ± 7.70 kg, for LSM vs. SFM, respectively). Larson et al. (2009) reported protein supplement offered to cows during late gestation resulted in increased birth weight compared to non-protein supplemented calves. Steer ADG was not different due to treatment (P = 0.18; 1.22 vs. 1.28 ± 0.03 kg, for LSM vs. SFM, respectively). Heifer birth wt and weaning wt were not affected by treatment (P > 0.45; 41.86 vs. 41.12 ± 1.53 kg; 240.30 vs. 250.96 ± 9.29 kg, for LSM vs. SFM, respectively). Stalker et al. (2006) found supplementation of 42% CP vs. no CP supplement prepartum in beef cows did not affect birth weight but calves born from supplemented cows had greater weaning weights. Conversely, Tou et al. (1998) reported lighter birth weights in rat offspring born from rat dams supplemented with 10% flaxseed. Overall heifer ADG was not significantly affected by supplementation (P = 0.84; 0.83 vs. 0.84 ± 0.05 kg, for LSM vs. SFM, respectively).

Puberty

We observed that nine heifers attained puberty before 6 mo of age (4 SFM and 5 LSM). Age at puberty (181.2 vs. 187.0 ± 7.0 d, for LSM vs. SFM, respectively) was not significantly affected by treatment (P = 0.58). Age at puberty was earlier than previously reported (Wiltbank et al., 1969, Martin et al., 1992, Martin et al., 2006). Tou et al. (1998) reported that 10% supplemented flaxseed in rat dam diets fed during gestation and lactation decreased age to puberty, lengthened the estrous cycle and resulted in persistent estrus in offspring. Due to the unanticipated early age of puberty measured at P_4 > 1.0 ng/ ml, conclusions cannot be made regarding treatment effects on actual date of puberty.

Summary

Linseed meal can be fed to beef cattle during late gestation without any negative effects on calf growth or reproductive development.

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Table 1. Late gestation cow ration and analyzed dietary composition.

Item	LSM	SFM
Ingredient	---Percent DM---	
Linseed Meal Pellet	9.7	-
Sunflower Meal Pellet	-	9.7
Light Barley	27.6	27.6
Straw	32.8	32.8
Corn Silage	29.9	29.9
Analyzed dietary nutrient content		
DM, %	88.93	89.45
CP, %	37.40	37.77
ADF, %	19.18	21.78
CF, %	15.34	17.43
NEm, / lb	0.81	0.82
NEg, / lb	0.53	0.53
Fat, %	3.21	3.82
Ca, %	0.43	0.42
P, %	0.99	1.04

[a] Supplements were offered at 2.2 kg/hd/d.
[b] Mineral was offered in ration to meet NRC (2000) recommended requirements.

Table 2. Steer calf performance from cows supplemented with LSM or control diet during last 60 d of gestation.

Item	LSM	SFM	St Error	P-value
Birth Date, Julian	91.0	87.3	2.58	0.41
Birth Wt, kg	43.99	44.76	1.42	0.72
Growth performance				
WWt, kg (d 170)	254.84	270.11	7.700	0.20
ADG, kg	1.22	1.28	0.032	0.18

Table 3. Heifer performance born from cows supplemented with LSM or control diet during last 60 d of gestation.

Item	LSM	SFM	St Error	P-value
Birth Date, Julian	93.2	89.2	2.42	0.32
Birth Wt, kg	41.86	41.12	1.53	0.74
Growth performance				
WWt, kg (d 170)	240.30	250.96	9.29	0.45
Wt Initial, kg (d 182)	247.89	259.00	8.30	0.38
Wt Mid, kg (d 226)	283.70	294.50	8.07	0.38
Wt End , kg (d 280)	321.00	332.20	8.41	0.38
ADG Initial, kg (d 170- 182)	0.54	0.58	0.12	0.83
ADG Mid, kg (d 182- 226)	1.14	1.20	0.10	0.68
ADG End, kg (d 226- 280)	0.59	0.60	0.08	0.97
Overall ADG	0.83	0.84	0.05	0.85
Heifer Age at Puberty				
Day of Age, Julian	181.2	187.0	7.01	0.58

[a] Day of age at puberty was determined when P_4 blood serum was > 1.0 ng/ml.

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EFFECTS OF ISOLATION ON SERUM AND SALIVARY CORTISOL AND COMPLETE BLOOD COUNTS IN EWES

D. T. Yates, T. T. Ross, A. R. Otis, C. A. Warner, L. J. Yates, and D. M. Hallford
New Mexico State University, Las Cruces, NM

ABSTRACT: Cortisol (C) has been used extensively as a physiological marker of stress in animals. The objective of this study was to compare changes in serum C, salivary C, and complete blood counts (CBC) in response to isolation and establish parameters for these physiological components in sheep. Twelve Suffolk ewes (10 mo of age, 64 ± 1.2 kg) were held indoors in a common pen or isolated pens for 10 d. Isolation pens established physical, but not visual or auditory isolation. Ewes were fed alfalfa hay (1.25 kg/ewe) daily at 0800. Serum and whole blood samples were collected via jugular venipuncture and saliva samples were collected via oral swab at 0700 and 1300 on each day (daily collections), and on the afternoons of d 1, 5, and 10, samples were taken in 15-min intervals for 2 h, beginning at 1300 (intensive collection). Serum and salivary C were determined by RIA. Serum and salivary C did not differ ($P > 0.05$) due to treatment and no treatment by sample period interaction was observed ($P > 0.05$). Isolation reduced ($P < 0.05$) hematocrit (Hct) and mean corpuscular volume, and increased ($P < 0.05$) mean corpuscular hemoglobin concentration. Red blood cells (RBC) were not affected ($P > 0.05$) by isolation. Likewise, total white blood cells, lymphocytes, neutrophils, basophils, eosinophils, and monocytes did not differ ($P > 0.05$) between treatments. The correlation coefficient between serum and salivary C was 0.83 ($P < 0.05$). Correlation coefficients between serum C and RBC, hemoglobin (Hgb), and Hct, were 0.45, 0.45, and 0.44, respectively ($P < 0.05$). All samples (daily and intensive) were used to calculate correlation coefficients. Data from this study indicate isolation for 10 d did not elicit strong or consistent release of C and, with few exceptions, did not alter components of CBC. However, changes in these parameters over time were observed, and strength of relationships between CBC components and C over the 10-d period was established.

Keywords: Complete blood counts, isolation, salivary cortisol, serum cortisol

INTRODUCTION

Analysis of stress influence on domestic animals requires safe, practical, and reliable methods of stress quantification. Specific components of the stress response such as stress hormone concentration, respiratory activity, and behavior are often indicators of stress (Rammerstorfer et al., 2001). However, cost, ease of sample collection, and accuracy, must be considered when selecting a stress marker. Serum cortisol (C) is used extensively as a marker in livestock research, where importance of stress management is governed by financial considerations. However, interest in a non-invasive sampling method has led to use of saliva as an alternative sample medium for C measurement (Cook and Jacobson, 1995). Additionally, non-acute stress can depress immune-related blood components, thus complete blood counts (CBC), which measure concentrations of these components, may also quantify stress (Davis et al., 2008). The objective of the current study was to compare changes in serum C, salivary C, and CBC components in response to isolation in sheep, and to establish parameters for these physiological components.

MATERIALS AND METHODS

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee (2008-024). Twelve yearling Suffolk ewes (64 ± 1.2 kg) were used to compare changes in serum and salivary C and CBC components in response to 10 d of physical isolation. Before use, ewes were weighed and physically examined for health. Six ewes were randomly assigned to a common 6 by 5 m indoor control pen (CON) and the remaining ewes were penned in individual 2 by 5 m indoor pens (ISO). All pens were constructed of 6 by 12 cm-square galvanized cattle panels, thus, ISO ewes were not visually or audibly obstructed from adjacent ewes. Isolation pens were spatially separated by no less than 2 m. Ewes were fed alfalfa hay (approximately 1.25 kg/ewe) once daily at 0800 and given *ad libitum* water. Approximately 7 cm of wood shavings were used as bedding over concrete floors. Bedding was changed every 72 h. Ewes were penned at 1200 on d 1 and remained until d 10. Blood serum, whole blood, and saliva samples were collected at 0700 and 1300 each day with the exception of d 1, on which no morning sample was collected. On d 1, 5, and 10, samples were intensively collected at 15-min intervals for 2 h, beginning at 1300.

Serum was collected via jugular venipuncture (Corvac serum separator tubes). Samples were kept at room temperature for 30 to 60 min and then centrifuged (1,500 x g at 4° C for 15 min). After centrifugation, samples were stored at -80° C until ready for assay. Radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA; Kiyma et al., 2004) was used to analyze C concentration in all samples (mean intra-assay CV = 2.5 % over 4 assays; inter-assay CV = 1.4 %). For each serum sample, a simultaneous saliva sample was collected. A 1 by 2 cm cotton strip was

held with hemostats and inserted into the mouth of the ewe. The ewe was allowed to chew on the cotton strip for 30 to 45 s before the strip was removed and placed in a specialized 10 mL centrifugable saliva collection device (Sarstedt salivette tube, Numbrecht, Germany). Salivettes were cooled immediately and centrifuged (1,500 x g at 4° C for 15 min) within 30 min of collection. Samples were frozen (-80° C) for storage while still cool. Cortisol concentration was determined via RIA as previously described (mean intra-assay CV = 3.6 % over 4 assays; inter-assay CV = 16.8 %). Whole blood samples were obtained by jugular venipuncture (EDTA-containing whole-blood vacuum tubes). Immediately after sampling, whole-blood samples were cooled and shipped overnight to the Veterinary Diagnostics Services, Albuquerque, NM, for CBC analysis: white blood cells (WBC), red blood cells (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets, and absolute counts of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils (BAS). On d 10, all ewes received a cautionary dose of liquamyosin (LA-200, 5 mL, s.c.).

Ewe was the experimental unit, and data were grouped for analysis into daily collections (samples collected twice daily at 0700 and 1300) or intensive collections (samples collected at 15-min intervals for 2 h on d 1, 5, and 10). Experimental design was a completely random design with repeated measures and treatment structure for all response variables was one-way with 2 classes: ISO and CON. All data were analyzed by SAS (SAS Inst. Inc, Cary, NC). Serum C, salivary C, and CBC data were analyzed for differences among treatments, differences among sample period, and treatment by period interactions via mixed procedure with repeated measures function. Diurnal expression of serum and salivary C was analyzed by comparison of daily samples collected at 0700 to those collected at 1300. Regression and correlation procedures of SAS were used to relate serum and salivary C to each other, as well as to each CBC component. Daily and intensive samples were pooled for regression and correlation analysis.

RESULTS

In daily samples, treatment by sample period interactions were observed ($P < 0.05$) for WBC, RBC, Hgb, Hct, MCV, MCHC, and NEU. When treatment was tested within period, WBC (Figure 1) was reduced ($P < 0.05$) in ISO ewes at 1300 on d 2 and 5 compared to CON, as were RBC (Figure 2) on d 2 and Hgb (Figure 3) on d 1 and 2. In ISO ewes, Hct (Figure 4) was reduced ($P < 0.05$) at 0700 on d 2, 3, 6, 9 , and 10, and at 1300 on d 1 to 5, 8, and 9, MCV (Figure 5) was reduced ($P < 0.05$) at 0700 on d 2 to 6 and at 1300 on d 2 to 7 and 9, and NEU count was reduced ($P < 0.05$) at 1300 on d 2 and 5, while MCHC (Figure 6) was greater ($P < 0.05$) at all sample periods except 0700 on d 2 compared to CON ewes. Interestingly, no effect ($P > 0.05$) due to treatment was observed for serum (Figure 7) or salivary C (Figure 8). All measured entities differed ($P < 0.05$) among sample periods except BAS counts.

In intensive samples, treatment by sample period interaction was observed ($P < 0.05$) for WBC, MCV, and MCHC. When treatment was tested within period, WBC in collection 1 and 4 of the second intensive period (d 5) and MCV in all collections of the second intensive were reduced ($P < 0.05$) in ISO ewes compared to CON, and MCHC in collections 2 through 8 of the first intensive period (d 1), all collections of the second intensive period, and collections 1, 2, and 6 of the third intensive period (d 10) were reduced ($P < 0.05$) in ISO ewes. No treatment by period interaction was observed ($P > 0.05$) for Hgb, Hct, or BAS counts, but all 3 were reduced ($P < 0.05$) in ISO ewes. As with daily samples, serum and salivary C did not differ ($P > 0.05$) between treatments. Differences ($P < 0.05$) among collection periods were observed for all entities except BAS count. In daily samples (Table 1), neither serum nor salivary C differed ($P > 0.05$) between samples taken at 0700 and those taken at 1300.

Table 1. Serum cortisol (ng/mL) and selected CBC components in samples taken at 0700 or 1300 daily for 10 days in ewes.

	Period			
Item	0700	1300	SE[1]	*P*-value
Serum cortisol	17.5	17.1	1.2	0.501
Salivary cortisol	0.5	0.6	0.1	0.592

[1]Standard error (n = 12).

Serum and salivary C were closely linked (r = 0.83; $R^2 = 0.69$; $P < 0.05$) to one another, but neither were strongly correlated with specific CBC components were not as strong (Table 2). These relationships ranged from moderate to very weak and, generally, relationships between salivary C and CBC components were slightly weaker than between serum C and the same components.

DISCUSSION

The lack of the isolation model implemented in the current study to induce changes in serum or salivary C indicates that physical isolation for 10 d without visual or auditory isolation was not a significantly strong stress to elicit a typical stress response. Minton et al. (1995) described a more complete stress response when isolation was coupled with restraint for 6 h/d for 3 d. It is unclear how this model would affect ewes over 10 d.

In general, immune components of CBC did not correlate well with serum or salivary C over the 10-d isolation period. This corroborates findings by Minton et al. (1995) that stress-related changes in these components were related to factors other than increased C. Yet, strong correlation between serum and salivary C supports our previous findings that saliva is a viable noninvasive alternative to serum for C analysis (Yates et al., 2008). Reduction of WBC by isolation at d 2 and 5 contradicted findings in surgically-stressed calves (Chase et al., 1995), which observed increased WBC at d 2. Additionally, reduction of Hct in ISO ewes differed from findings of no change due to transportation stress (Averós et al., 2008). Changes in Hct and RBC were deemed by Averós et al. (2008) to be related to dehydration associated with

physical stress on the body and, thus, might be more representative of physical exertion than C or WBC. Gupta and Flora (2005) described LYM concentration and MCV as markers of long term stress, while MON concentration increased with stress of shorter duration. However, MCV in the current study changed in ISO ewes as early as d 2, while LYM differed in only a small number of collections in the d 1 intensive and were not affected by isolation thereafter. Generally, isolation appeared to most profoundly affect Hct, MCV, and MCHC in daily samples and Hgb, Hct, and MCHC in intensive collections. This may have been due to dehydration associated with unrest caused by isolation. Diurnal rhythms in C reported in samples taken 12 h apart in dairy cows (Lefcourt et al., 1993) were not observable at 6 h in the current study.

A single, universal marker of physiological stress has yet to be established. In fact, evidence from this and previous studies indicate that both magnitude and direction of changes in specific proposed markers in response to stress depend strongly upon type, intensity, and duration of stress. Future inquiries should concentrate upon defining specific situations for which individual markers would be most suited, so that maximum value may be obtained from each. Accordingly, the current study indicates that Hgb, Hct, MCV, and MCHC may change with mild isolation.

Table 2. Regression (R^2) and correlation (r) coefficients between serum or salivary C and CBC components in samples collected in ewes.

	Serum C		Salivary C	
Item	R^2	r	R^2	r
Serum C	.	.	0.69	0.83
Salivary C	0.69	0.83	.	.
WBC	0.00[1]	0.02[1]	0.00[1]	0.02[1]
RBC	0.25	0.45	0.13	0.35
Hgb	0.24	0.45	0.14	0.36
Hct	0.23	0.44	0.12	0.34
MCV	0.01[1]	0.07[1]	0.00[1]	0.07[1]
MCHC	0.04	-0.17	0.02	-0.13
Platelets	0.05	-0.23	0.04	-0.19
NEU[2]	0.18	0.41	0.15	0.36
LYM[2]	0.21	-0.43	0.16	-0.37
MON[2]	0.10	0.31	0.08	0.26
EOS[2]	0.10	-0.30	0.10	-0.30
BAS[2]	0.01	0.10	0.02	0.12
A_NEU[3]	0.09	0.26	0.07	0.22
A_LYM[3]	0.02	-0.16	0.02	-0.14
A_MON[3]	0.09	0.28	0.08	0.27
A_EOS[3]	0.06	-0.23	0.07	-0.26
A_BAS[3]	0.01	0.09	0.02	0.11

[1]($P > 0.05$).
[2]Expressed as fraction of total WBC.
[3]Total counts.

IMPLICATIONS

Isolation of ewes for 10 d apparently did not prove sufficiently stressful to elicit changes in the traditionally used stress marker, cortisol. Isolation caused changes in Hgb, Hct, MCV, and MCHC, possibly due to unrest or dehydration. Relationships between cortisol and specific immune components were generally weak, indicating that stress influence on immune function was independent of cortisol. Changes between morning and early afternoon levels were not observed in serum or salivary cortisol indicating that diurnal changes detectable at 12 h were not detectable at 6 h.

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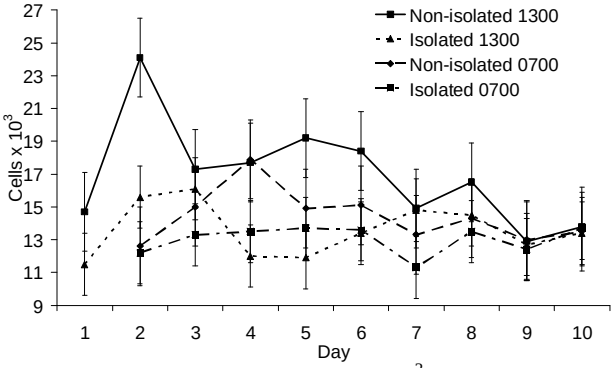

Figure 1. White blood cells (cells x 10^3) in whole blood samples collected at 0700 or 1300 in isolated and non-isolated ewes (treatment by sample period, $P < 0.05$).

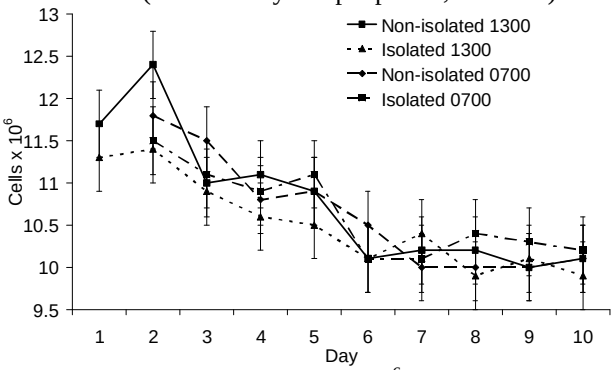

Figure 2. Red blood cells (cells x 10^6) in whole blood samples collected at 0700 or 1300 in isolated and non-isolated ewes (treatment by sample period, $P < 0.05$).

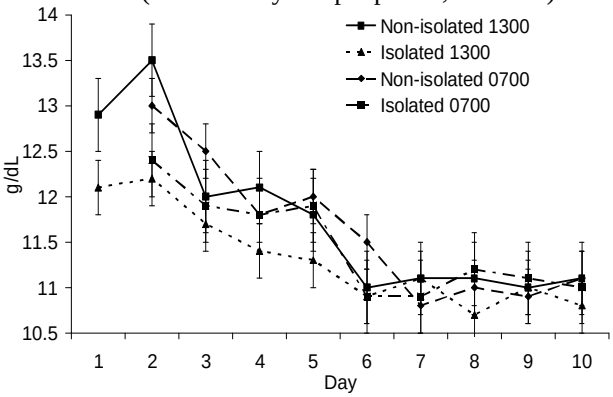

Figure 3. Hemoglobin (g/dL) in whole blood samples collected at 0700 or 1300 in isolated and non-isolated ewes (treatment by sample period, $P < 0.05$).

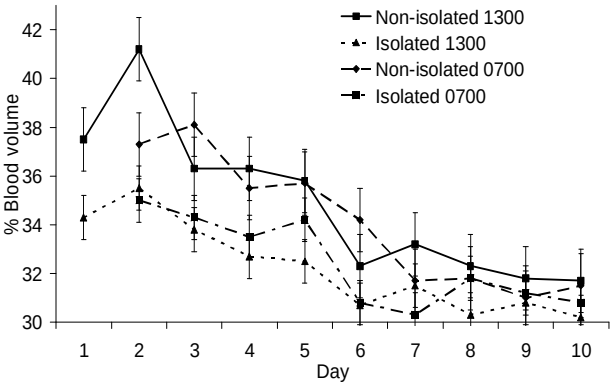

Figure 4. Hematocrit (%) in whole blood samples collected at 0700 or 1300 in isolated and non-isolated ewes (treatment by sample period, $P < 0.05$).

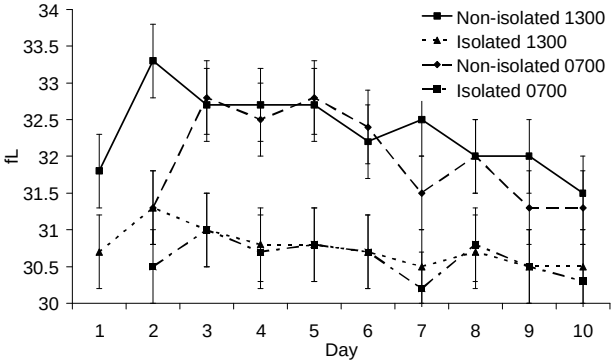

Figure 5. Mean corpuscular volume (fL) in whole blood samples collected at 0700 or 1300 in isolated and non-isolated ewes (treatment by sample period, $P < 0.05$).

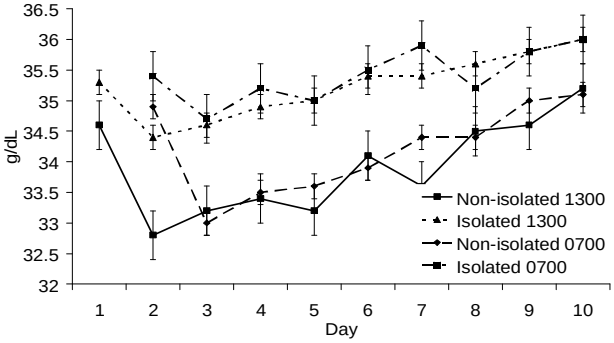

Figure 6. Mean corpuscular hemoglobin concentration (g/dL) in whole blood samples collected at 0700 or 1300 in isolated and non-isolated ewes (treatment by sample period, $P < 0.05$).

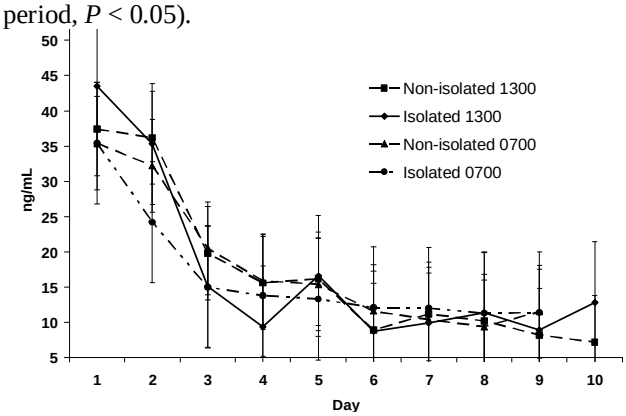

Figure 7. Cortisol (ng/mL) in serum samples collected at 0700 or 1300 in isolated and non-isolated ewes (treatment by sample, $P > 0.05$).

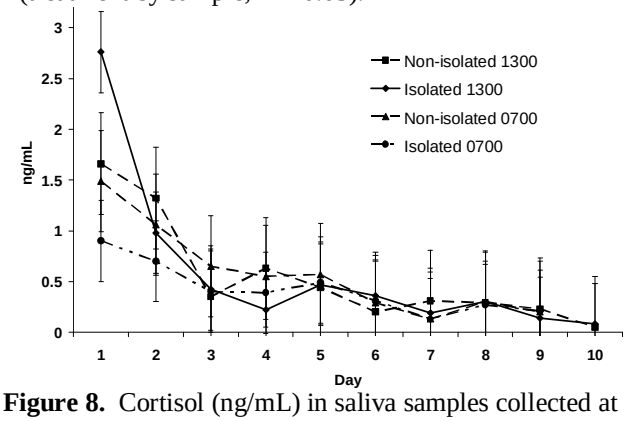

Figure 8. Cortisol (ng/mL) in saliva samples collected at 0700 or 1300 in isolated and non-isolated ewes (treatment by sample, $P > 0.05$).

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LATE GESTATIONAL NUTRITION OF THE MARE AND SUBSEQUENT EFFECTS ON ADRENAL FUNCTION OF THE OFFSPRING AS A YEARLING

S. A. Burns[1], C. J. Hammer[2], J. A. Coverdale[1], C. A. Cavinder[1]; Texas A&M University, College Station, TX USA[1], North Dakota State University, Fargo, ND USA[2]

ABSTRACT: The present study aims to determine if maternal nutrition of the mare during the last trimester of gestation has an effect on the adrenal function of the resulting offspring at a later point in life.

Quarter horse yearlings (n=9) who were foaled from mares either fed on pasture alone (P; 100% NRC requirements) or fed pasture and grain (PG; 120% NRC requirements) during the last trimester of gestation were evaluated for adrenal function. Yearlings were randomly assigned to 1 of 2 groups (ACTH treatment or saline control) and then switched back so that each horse was used as a treatment and a control. Blood samples were collected periodically around treatment with either ACTH or saline and later analyzed for concentrations of cortisol.

The results of this study revealed no statistical difference in the strength or duration of the cortisol response regardless of maternal nutrition, P or PG. When treated with ACTH, peak cortisol concentrations were observed at 90 min post-treatment, with a notable decrease in concentration occurring between 90 and 150 min post-treatment. Cortisol concentration at 150 min was still elevated from baseline ($P<0.05$).

The current study suggests that nutritional status of the mare does not affect adrenal function of the subsequent yearling when the mare is being fed at least 100% of NRC requirements. Additionally, this study provides information on doseage requirements for evaluating adrenal response in the yearling along with suggesting adrenal response time.

Keywords: mare, cortisol, adrenal function, foal

Introduction

There is significant evidence to suggest that changes in maternal nutrition during gestation impacts the programming of the fetal hypothalamic-pituitary-adrenal axis (Bloomfield et al., 2003; Challis et al., 2001; Moore and Davies, 2005; McMillen and Robinson, 2001). Moore and Davies (2005) raised questions about maternal nutrition composition, suggesting that it is not only the quantity of nutrition but also the composition of the maternal diet that determines fetal health during gestation. Fowden (2006) also documented that maternal stress and nutrition, even when not associated with decreased birth weights, caused alterations in cardiovascular and metabolic function in the resulting offspring. Given that birth size may not be a reliable indicator of fetal health and metabolic function, more emphasis must be placed on maternal nutrition and diet composition throughout pregnancy.

Adrenocorticotropic hormone (ACTH) stimulates the adrenal glands to release glucocorticoids, including cortisol. In an ACTH challenge, a synthetic form of ACTH is administered to elicit an adrenal response. Circulating levels of cortisol can be measured to determine the strength of the adrenal response. Excessively low or high adrenal secretion is considered dangerous and can contribute to significant metabolic disorders.

While evidence has shown that maternal nutrition can affect the programming and maturation of fetal systems including the HPA axis, the effect on offspring later in life has not yet been reported in horses.

Materials and Methods

Background

In a 2007 study, 28 Quarter Horse mares were used to assess the effect of late-gestational nutrition on the neonatal foal. Forty-five days prior to the last trimester of gestation, half of the mares were assigned to a group which was fed grain at 0.75% BW (PG), while the remaining mares were kept on pasture alone (P). As a result of this protocol, the PG mares were fed at 120% of their NRC requirement, while the P group was maintained at 100% of their NRC requirement.

ACTH Challenge

Yearlings (n=9) from mares used in the initial 2007 study were retained by Texas A&M University and used in an ACTH challenge. Four of the yearlings were from mares fed at 100% NRC during their last trimester of gestation, and 5 were from mares fed at 120% NRC. The yearlings were randomly assigned to 1 of 2 groups (treatment with ACTH or control). After one week, the groups were reversed and the challenge was repeated so that each yearling participated in the study as both a control and a treatment animal.

For this study Cortrosyn® (Amphastar Pharmaceuticals, Rancho Cucamonga, CA) a brand of the synthetic ACTH cosyntropin, was administered to generate the adrenal response. Blood samples were obtained by jugular venipuncture from each animal at -30, 0, 30, 60, 90 and 150 minutes. At time 0, blood was obtained from each horse and then horses in the treatment group received an i.v. injection of 0.25mg of cosyntropin reconstituted with 1.0 mL of a 0.9% sodium chloride solution. At the same time,

horses in the control group were administered 1.0 mL of 0.9% sodium chloride alone. Blood samples were collected into heparinized tubes and then centrifuged at 2500 rpm for 20 min at 4°C in a refrigerated centrifuge. Plasma was removed, labeled and stored at -20°C until cortisol RIA analysis (Seimens Medical Solutions Diagnostics, Los Angeles, CA) previously validated for horses (Hedberg et al., 2007).

Stastistical Analysis

Mean plasma cortisol concentrations were analyzed by 1-way ANOVA. Group, time and group by time interactions were evaluated.

Results

Between group differences in adrenal response

Mean control (Fig. 1) and treatment (Fig. 2) values for the 2 groups of yearlings were compared, and were not different ($P>0.05$) at any time point throughout the challenge. The adrenal responses for the 2 groups of horses were comparable, despite the mares' different planes of nutrition during gestation.

Overall response to adrenal stimulation

When comparing control to treatment values (Fig. 3), treatment values show an elevation in cortisol for all animals regardless of group (P vs. PG). Furthermore, when horses were treated with ACTH, peak cortisol concentrations were observed at 90 min post-treatment, with a notable decrease in concentration occurring between 90 and 150 min post-treatment. Cortisol concentration at 150 min was still elevated from baseline ($P<0.05$), indicating that it takes more than 150 min for peripheral cortisol concentrations to return to pre-stimulation levels. The small administration dose of 0.25mg of synthetic ACTH proved to be an effective dosage for eliciting a significant adrenal response in yearlings.

The results of this study revealed no statistically significant difference in the strength or duration of the cortisol response in these yearlings regardless of group, P or PG; thus, the observed baseline and threshold cortisol values of the yearlings did not appear to be related to the nutritional status of their respective mothers (P vs. PG).

Discussion

Slight over-feeding of mares during the late pre-partum period does not seem to affect adrenal function and, thus, cortisol response to an ACTH challenge in the offspring of horses up to 18 mo of age. Furthermore, the yearlings all had comparable baseline values when treated with saline and used as controls.

A recent study of the endocrine profile for mares and foals suggested that leptin concentrations may be depressed in horses up to 2 yrs of age (Berg et al., 2007). While no study has been published as to the degree of variation in cortisol response in young horses, it is plausible that the cortisol response is similarly variable in these animals until they reach puberty. Perhaps, effects of maternal nutrition on cortisol response and feedback mechanisms in the offspring take time to develop and would be more prevalent in older animals.

In sheep, it has been documented that the duration of nutritional stress plays an important role in the long-term consequences of fetal programming. In nutrient restricted ewes, a significant alteration in HPA function of the offspring was observed when nutrition was restricted for a period of 10 d, but not when the restriction persisted for 20 d. It was suggested that brief undernutrition causes a differential programming of the fetal HPA axis which persists into adulthood. On the other hand, the longer period of nutritional stress may allow the fetus more time to adapt to the uterine environment and lead to fewer consequences in the adult (Bloomfield et al., 2003).

While maternal nutrition and stress clearly help shape the development and programming of fetal metabolic and regulatory systems, there is also evidence that some additional programming may occur post-parturition. Maternal milk components, including IGF-1 and thyroid stimulating hormone, may aid in the development of proper metabolic function in the neonate (Berg et al., 2007). In foals, research has shown that some additional maturation and development of the HPA axis and hormonal control mechanisms occur post-parturition and continues through the early life of the foal (Hart et al., 2007). In addition, post-natal manipulation and interaction with the mother and/or humans has been shown to reverse some effects of prenatal stress and contribute to proper metabolic function in rats (Welberg and Seckl, 2001). These observations support the idea that maternal undernutrition and/or stress only contribute to part of the programming of metabolic systems. There are many other intrinsic and extrinsic factors that may influence the development and proper functioning of adult metabolism and regulatory processes. With that said, the current study did not feed mares under what is recommended by NRC for late gestating mares, however, compared maintenance type diets to mares that were overfed. Perhaps if BCS was the main determinant to assess nutritional status (i.e. mares in a BCS of 7 versus 3) a difference in foal birth size and potentially other factors later in the life of the offspring would be detected.

Maternal nutrition status and fetal exposure to glucocorticoids *in utero* have been shown to have lasting effects on health of the adult offspring. Several studies have demonstrated a positive correlation between elevated cortisol levels and high blood pressure in offspring whose mothers were stressed during gestation (Phillips et al., 2005; Emack et al., 2008). In humans, prolonged elevated plasma cortisol concentration is associated with glucose intolerance, insulin resistance and high blood pressure, symptoms which can indicate increased risk for cardiovascular complications in adulthood (Emack et al., 2008). Horses with altered adrenal function due to maternal nutrition and poor uterine environment may also experience similar complications. Elevated baseline and threshold cortisol values may affect the animal's ability to respond to external stressors and recover from illness. Extreme

hypercortisolism is a characteristic of Cushing's Syndrome (Singh, 2006). Horses that display chronic elevation in cortisol levels could potentially experience serious health complications including diabetes and heart disease, potentially reducing their functionality and usefulness.

More research into the effect of timing and duration of maternal stress and nutrition on fetal programming must be conducted to determine the extent to which the uterine environment affects the later health of the offspring. Furthermore, the maturation of hormonal and metabolic control mechanisms clearly shows some species specificity, so more must be learned about programming mechanisms in the equine before assumptions can be made about the lasting effect that maternal nutrition will have on the offspring.

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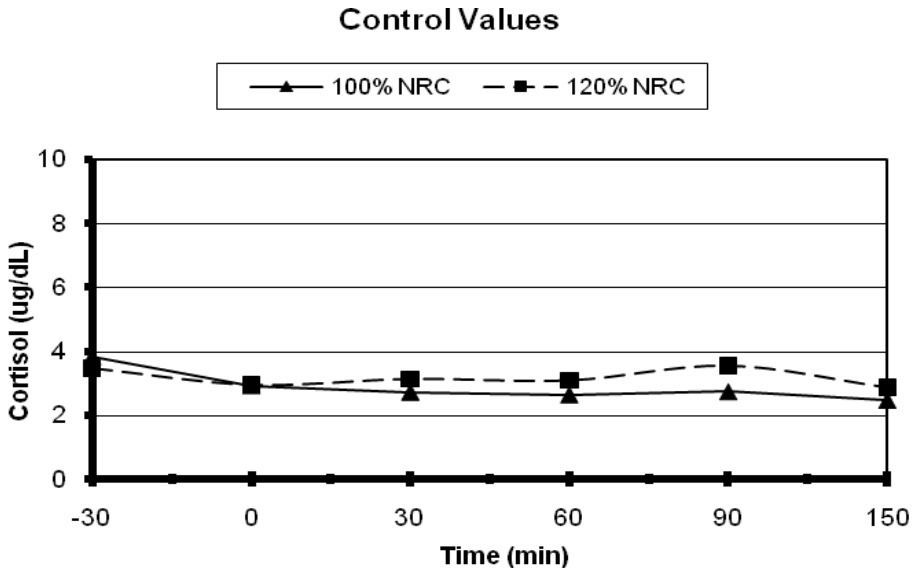

FIG. 1. Mean circulating plasma concentrations of cortisol after administration of 1.0 mL saline at time 0 for yearlings foaled from mares fed either 100% or 120% NRC requirements during the last trimester of gestation.

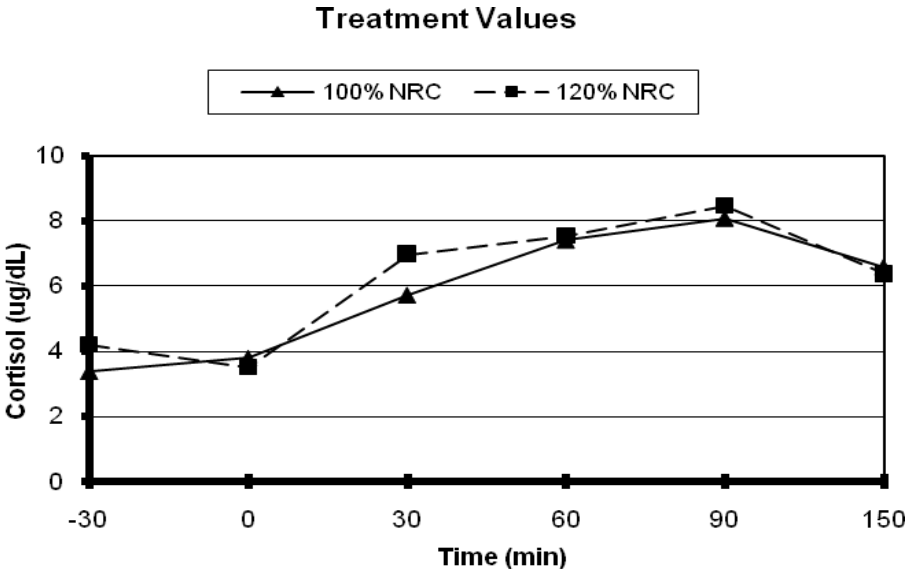

FIG. 2. Mean circulating plasma concentrations of cortisol after administration of 0.25 mL ACTH at time 0 for yearlings foaled from mares fed either 100% or 120% NRC requirements during the last trimester of gestation.

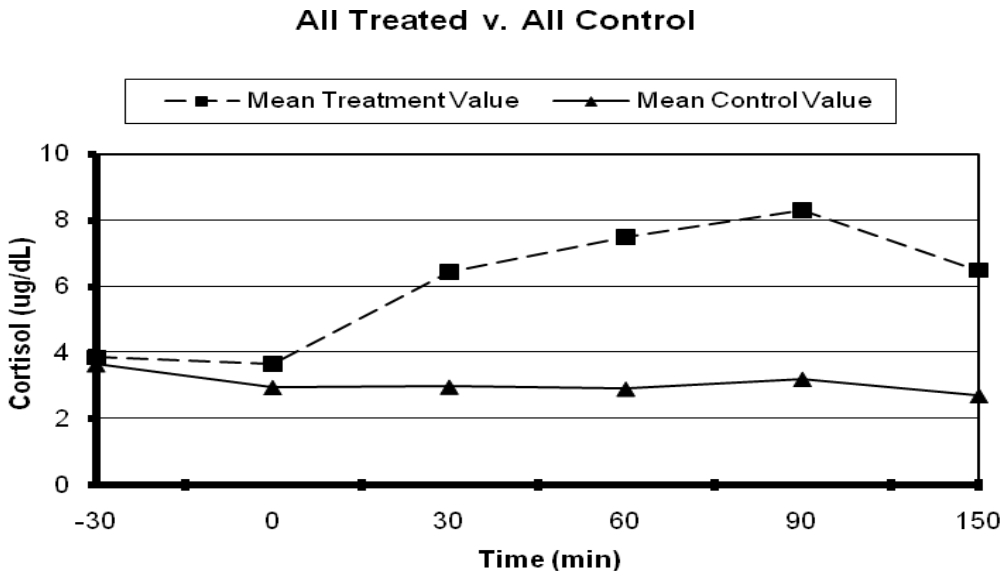

FIG. 3. Mean circulating plasma concentrations of cortisol after administration of 1.0 mL saline or 0.25 ACTH at time 0 for yearlings yearlings (n=9) foaled from mares fed either 100% or 120% NRC requirements during the last trimester of gestation.

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FASTING LOWERS GASTRIN-RELEASING PEPTIDE AND FSH mRNA IN THE OVINE ANTERIOR PITUITARY GLAND

A. M. Fuller[1], K. J. Austin[1], A. J. Roberts[2], G. E. Moss[1], and B. M. Alexander[1]
Dept. of Animal Science, University of Wyoming, Laramie[1], USDA-ARS, Miles City, MT[2]

ABSTRACT: Estrogen receptor beta (ER-β), LH, and FSH are important mediators of reproduction. Follicle recruitment and development is stimulated by FSH. During anorexia, serum concentrations of FSH and LH decrease. Gastrin-releasing peptide (GRP), neuromedin B (NMB), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) and thyroid-stimulating hormone (TSH) are important metabolic regulators expressed in the anterior pituitary gland (AP). Gastrin-releasing peptide stimulates release of ACTH, is associated with melanocortin in regulating food intake, and is a regulatory peptide in the female reproductive tract. In cattle, pituitary GRP expression was markedly up-regulated after resumption of estrus following parturition, indicating a connection between gene expression of GRP and reproductive function. The objective of this study was to determine effects of fasting during the luteal phase of the estrous cycle on gene expression in the anterior pituitary gland during the subsequent periovulatory period. Estrus was synchronized in mature (≥ 3 yr old) western white-faced ewes with prostaglandin $F_2\alpha$ ($PGF_2\alpha$). Randomly selected ewes were fed grass hay ad libitum (control = 10) or were withheld from feed on days 7 – 11 of their estrous cycle (d 1 = estrus; fasted = 10). On d 12, fasted ewes were returned to feed and all ewes were treated with $PGF_2\alpha$ (0 hrs). Pituitaries were collected 72 h after $PGF_2\alpha$. Ovaries were observed for presence of pre-ovulatory follicle or newly formed CL. Pituitaries were analyzed (n = 5 each group) from ewes that had ovulated. Fasting decreased ($P < 0.05$) gene expression of GRP and FSH. Differences in gene expression were not noted ($P \geq 0.26$) in mRNA levels of PGC-1α, TSH, NMB, ER-β, or LH. Mediation of metabolic effects on reproductive function may be regulated by GRP affecting expression of FSH.

Key Words: Fasting, Pituitary, GRP, FSH.

Introduction

Research continues to determine how food intake and metabolism effect reproduction. Factors known to mediate food intake are gastrin-releasing peptide (GRP), neuromedin B (NMB), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α), and thyroid-stimulating hormone (TSH). Bombesin-like peptides, GRP and NMB, have anorexigenic effects (Frank, 2001; Polya, 2003). Production of TSH from the anterior pituitary gland is directly acted upon by GRP. Along with metabolic effects, GRP stimulates the release of LH from AP cells suggesting a role in mediating reproductive function (Evans, 1999). Immunoreactive GRP is found in mature lactotrophs, corticotrophs, and immature somatotrophs, thyrotrophs, and gonadotrophs in the rat. (Houben, 1991).

A major regulator of mitochondria, PGC-1α, affects metabolic homeostasis and, possibly, cellular energy expenditure (Cantó, 2009). It also plays a role in reproduction by interacting with estrogen receptor alpha to produce progesterone in rat ovarian granulosa cells (Chen, 2008). Other genes important in reproductive function are those encoding for FSH, LH, and estrogen receptor beta (ER-β). In addition to reproductive effects, ER-β mediates glucose metabolism (Foryst-Ludwig, 2008). Luteinizing hormone and FSH are responsive to changing metabolism and body condition. Serum concentrations of FSH and LH are decreased in anorexic individuals (Tomova, 2007).

The objective of this study was to determine if fasting during the luteal phase in mature female ewes affected anterior pituitary gland levels of mRNA for ER-β, LH, FSH, GRP, NMB, PGC-1α, and TSH during the subsequent periovulatory period.

Materials and Methods

Animals,Treatment. Mature (≥ 3 yr old) western white-faced ewes were synchronized with two 10 mg doses of $PGF_2\alpha$ (Lutalyse, Pharmacia &Upjohn Co., Kalamazoo, MI) on d 1 and d 10 (d 1 = first day of estrous). Following the injection of $PGF_2\alpha$ on d 10, estrous behavior was monitored with two vasectomized rams for 4 d. Ewes with synchronized estrous cycles (n = 20) were randomly allotted to control (n = 10) or fasted (n = 10) groups. Ewes were housed separately by treatment in adjacent pens. Control ewes were fed grass hay ad libitum. Fasted ewes were withheld from feed on d 7 to 11 of their estrous cycle. On d 12, fasted ewes were returned to feed and all ewes were given a 10 mg injection of $PGF_2\alpha$. Pituitaries and ovaries were collected 72 h after $PGF_2\alpha$ administration. Pituitaries from ewes that ovulated (n = 5 each group) were snap frozen for analysis.

RNA Isolation and cDNA Synthesis. Approximately 100 mg of anterior pituitary gland tissue from each animal was homogenized in 1 mL of TRI reagent (Sigma Aldrich Chemical; St. Louis, MO). Concentrations of RNA were determined using a NanoDrop spectrophotometer. Then RNA was purified using an RNEASY kit (Qiagen Inc; Santa Clara, CA). A 20 µL reaction using 4 µL reverse transcription buffer (5X), 1 µL of IScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA) and 2.0 µg RNA was used to

synthesise cDNA. The thermocycler program ran for 5 min at 25 ºC, 30 min at 42 ºC, 5 min at 85 ºC and held at 4 ºC. Synthesized cDNA was diluted 6-fold with nuclease-free water and stored at -20 ºC.

Semi-Quantitative Real Time RT-PCR. Diluted cDNA was mixed with SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), nuclease-free water, and a forward and reverse primer. Primer3 software was used to design primers for ovine ER-β, LH, FSH, GRP, PGC-1α, TSH, and bovine NMB. Semi-quantitative RT-PCR was performed using 40 cycles of 95 ºC for 30 sec and 1 cycle of 62 ºC for 30 sec. Following amplification, cDNA were melted to ensure quality of amplification by incubating RT-PCR products for 10 sec at each step with an increase in temperature by 0.5 ºC from 55 ºC to 95 ºC in each cycle. All gene expression levels were quantified relative to GAPDH.

Statistical Analysis. All mRNA data were analyzed by SAS (Version 9.0). A one-tailed t-test was used to determine mean differences in the average fold change of mRNA expression within the anterior pituitary glands of fasted ewes compared to control ewes.

Results

Gene expression of GRP ($P = 0.03$) and FSH ($P = 0.04$) were down-regulated in the anterior pituitary gland of fasted ewes compared to control ewes (Fig. 1). Expression of mRNA for ER-β, LH, PGC-1α, TSH and NMB did not differ ($P \geq 0.26$) between treatments (Table 1).

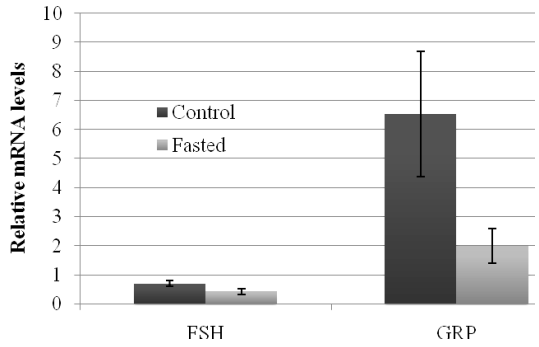

Figure 1. Expression of FSH and GRP mRNA in the anterior pituitary gland of control and fasted ewes. ($P < 0.05$)

Table 1. Relative concentrations of mRNA in the anterior pituitary gland of fasted compared to control ewes.

Gene	Fold Change	*P* value
ER-β	1.13	0.34
LH	0.84	0.26
PGC-1α	1.11	0.36
TSH	1.13	0.42
NMB	0.93	0.41

Discussion

Differences in ER-β levels were expected because ER-β is a mediator of insulin/glucose metabolism and previous studies demonstrated decreased insulin during fasting (Kiyma, 2004). The glucose/insulin system is dynamic and changes rapidly, suggesting ER-β mRNA levels may have readjusted following fasting.

The lack of food in the stomach from fasting could cause an increase in orexigenic hormones and a decrease in anorexigenic hormones. Ruminant animals, such as sheep, have a slower rate of digestive passage than non-ruminants which could suggest that effects of fasting on anorexigenic hormones may be less acute than in non-ruminants. Such a delay in the need for anorexigenic hormones may relate to decreased GRP gene expression in the AP.

A decrease in TSH and LH would be expected since low doses of synthetic porcine GRP injected into male rats stimulated LH release and suppressed TSH secretion (Güllner, 1983). The lack of differences in the current study could be associated with the cyclicity of females or may reflect differences among species. Alternatively GRP may regulate release of TSH and LH but not regulate its synthesis.

Levels of FSH decrease with long term anorexia. This decreased level of FSH, along with LH can cause women to experience amenorrhea. While LH increases rapidly FSH levels gradually return to normal following food intake in anorexic individuals. The pathway involved in regulating this change in reproductive function is relatively unknown.

Implications

This study suggests GRP may play a role in mediating nutritional induced changes in reproductive function through FSH. Since both FSH and GRP are produced by AP gonadtrophs, the current results provide support for an autocrine / paracrine mechanism of regulation.

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BEEF COW PERFORMANCE FOLLOWING RUMEN-PROTECTED CHOLINE SUPPLEMENTATION DURING THE PREPARTUM PERIOD

J. R. Jaeger[*], K. C. Olson[†], S. R. Goodall[‡], and J. W. Bolte[*]

[*]Western Kansas Agricultural Research Centers, Kansas State University, Hays, KS 67601, [†]Kansas State University, Manhattan, KS 66506, [‡]Feed Industries Consultant, Erie, CO 80516

ABSTRACT: We determined the effects of prepartum rumen-protected choline (RPC) supplementation on beef cow performance. Angus x cows (n = 144) were stratified by age, BW, and body condition score (BCS), and assigned randomly to: control (CON) or RPC. Cows were maintained in separate groups, and received ad libitum forage sorghum hay and 0.95 kg/hd/d ground milo containing a trace mineral supplement; RPC was added to the grain supplement to provide 4 g/hd/d choline for RPC-treated cows. Supplementation occurred for 40 d and began 40 d before expected onset of calving. Backfat (BF) at the 13th rib, marbling score (MB), and longissimus muscle depth (LMD) were measured via ultrasound. Cow BW and ultrasound measurements were collected at start and end of supplementation. Cow BCS was recorded at start of supplementation and at calving. Response variables were analyzed by ANOVA. Reproductive responses were: proportion cycling at estrous synchronization; fixed-time AI (FTAI) conception rate; anestrous cow FTAI conception rate; and final pregnancy rate. These data were analyzed by the CATMOD procedure of SAS. Weight change and ADG during supplementation were not affected ($P > 0.70$) by treatment. Treatment did not affect ($P > 0.20$) change in BF, MB, or LMD and averaged -0.29 ± 0.11 mm, 1.87 ± 0.07, and -2.75 ± 0.77 mm, respectively. Change in BCS (-0.77 ± 0.05) from start of supplementation to calving was similar ($P = 0.66$) between treatments. Birth date and calf BW at birth was not different ($P > 0.70$) between treatment groups. Proportion of estrual cows (59%) at synchronization was similar ($P = 0.82$) between treatments. Conception to FTAI tended ($P = 0.13$) to be greater for RPC (58.1%) than CON (45.7%). Proportion of anestrous cows conceiving to FTAI was not different ($P = 0.37$), but numerically greater for RPC (58.1%) vs. CON (46.4%). Final pregnancy rate did not differ ($P = 0.46$) between treatments. These data were interpreted to suggest that prepartum supplementation with RPC had no effect on cow body condition but tended to improve conception to AI.

Key Words: Beef Cows, Choline, Reproduction

Introduction

Choline (Vitamin B4) is a precursor of acetylcholine and phosphatidylcholine, and is a methyl group donor through conversion to betaine. Methyl groups are required for a variety of metabolic reactions including methionine recycling and fatty acid mobilization.

Choline is rapidly degraded in the rumen (Sharma and Erdman, 1988). Therefore ruminants have limited intestinal absorption of methyl groups due to ruminal degradation of choline and betaine.

Supplementation of dairy cows with rumen-protected choline (**RPC**; 12 g/hd/d) from 28 d prepartum to 63 d postpartum resulted in increased milk yield and accelerated body weight loss after calving (Hartwell et al, 2000). Increasing dietary RPC in periparturient dairy cows also resulted in increased liver glycogen and liver esterified lipids secretion (Piepenbrink and Overton, 2003). In contrast, Janovick Gueretzky and coworkers (2006) observed no production or metabolic benefits from feeding 15 g/hd/d RPC to dairy cows pre- and postpartum.

Supplementing beef cows with RPC during the peripartum period tended to increase ADG; however, supplementation during the postpartum period resulted in accelerated weight loss similar to previous reports for dairy cows (Jaeger et al., 2008). Although cows receiving RPC during the periparturient period lost more weight than control cows, a greater proportion of RPC-treated cows tended to conceive to fixed-time AI (Jaeger et al., 2008). If postpartum weight loss could be avoided only by feeding RPC prepartum perhaps first service conception rates can be further enhanced.

Our objective was to determine if providing RPC to beef cows during the later part of the third trimester could improve first service conception rate to fixed-time AI.

Materials and Methods

Animals, Treatments and Diet. Procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Angus-cross cows (n = 144; age = 3 to 11 yr) were stratified by age, BW, and body condition score (BCS; 1 = emaciated, 9 = very obese; Wagner et al., 1988) and assigned randomly to one of two prepartum supplementation treatment groups: control (**CON**) or rumen-protected choline (**RPC**). Cows were maintained in separate groups and received *ad libitum* forage sorghum hay and 0.95 kg/hd/d ground milo containing a trace mineral supplement. Supplement contained rumen-protected choline (4 g/hd/d choline) and SQM trace mineral (Quali Tech, Chaska, MN) for RPC-treated cows and SQM trace mineral only for control cows (Table 1). Supplementation occurred for 40 d and began 40 d before expected onset of calving.

Table 1. Supplement composition

Ingredient, % DM	Treatment group Control	RPC
Rolled milo	69.05	69.05
Soybean meal	25.00	25.00
Trace mineral supplement (5.95 %)		
Zinc	0.08	0.08
Manganese	0.08	0.08
Copper	0.03	0.03
Rumen-Protected Choline	0.00	0.54

Data Collection. Cow BW and ultrasound measurements were collected at the beginning and end of supplementation. Backfat (**BF**) thickness, marbling (**MB**), and longissimus muscle depth (**LMD**) were measured in the region of the 12th and 13th ribs via ultrasound using an Aloka 500V (Aloka Co., Ltd, Wallingford, CT) B-mode instrument equipped with a 3.5-MHz general purpose transducer array (UST 5021-125 mm window). Images were collected with Cattle Performance Enhancement Company (**CPEC**, Oakley, KS) software. Backfat thickness, LMD, and MB were estimated with procedures that incorporated image analysis software (Brethour, 1994) that are an integral component of the CPEC product. Marbling scores were coded such that 4.0 = slight^{00} (low select) and 5.0 = small^{00} (low choice). Cow BCS was recorded at start of supplementation and at calving.

Estrous Synchronization and Breeding. Cows were stratified by supplementation treatment, age, and calving date and assigned randomly to a control or treatment synchronization group. Cows in the control group (7dCIDR) received GnRH (2 ml Fertagyl, i.m.) and an intravaginal insert containing 1.38 g of progesterone (EAZI-BREED CIDR) on d -7, CIDR removal and $PGF_{2\alpha}$ (2 ml Estrumate) on d 0, and fixed-timed AI and GnRH at 56 h after $PGF_{2\alpha}$ (Figure 1; Dobbins et al., 2006).

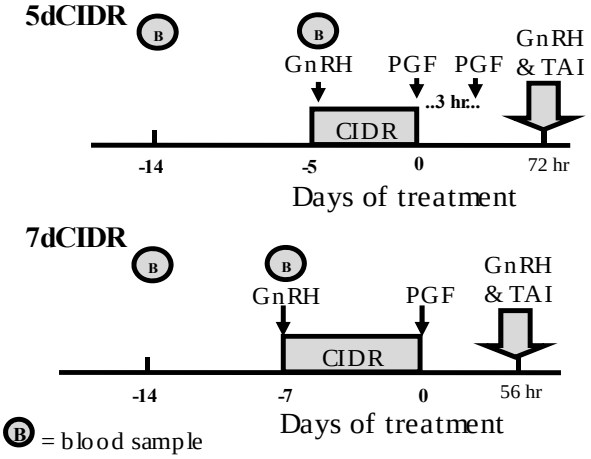

Figure 1. Estrous synchronization treatments and blood sampling schedule. TAI=timed AI.

Treated cows (5dCIDR) received GnRH and CIDR on d -5, CIDR removal and two injections of $PGF_{2\alpha}$ (2 ml Estrumate per injection) 3 h apart on d 0, and fixed-timed AI concurrent with GnRH at 72 h after $PGF_{2\alpha}$ (Figure 1). Bulls were introduced 10 d after fixed-timed AI. Pregnancy rate to AI was determined 31 d after fixed-time AI with transrectal ultrasonography. Cows grazed native pasture for 1.5 mo prior to the start of the breeding season and remained in one group until d -7. Cows were combined into a single group again following fixed-time AI.

Two serum samples were collected 9 or 7 d before and at the start of treatment for 5dCIDR and 7dCIDR, respectively, for measurement of progesterone by RIA (Skaggs et al., 1984; Figure 1). When one or both samples contained concentrations of progesterone ≥ 1 ng/ml cows were considered to be cycling.

Statistics. Response variables for cow performance were analyzed as a completely-random design. Reproductive responses were: proportion cycling at estrous synchronization; fixed-time AI conception rate; cycling cow fixed-time AI conception rate, anestrous cow fixed-time AI conception rate; and final pregnancy rate. There was no interaction between prepartum supplementation treatment and estrous synchronization system; therefore estrous synchronization system was removed from the final model. These data were analyzed by the CATMOD procedure of SAS.

Results

Weight change and ADG during supplementation were not affected ($P > 0.70$) by treatment. Treatment did not affect ($P > 0.20$) change in BF, MB, or LMD and averaged -0.29 ± 0.11 mm, 1.87 ± 0.07, and -2.75 ± 0.77 mm, respectively. Change in BCS (-0.77 ± 0.05) from the start of supplementation to calving was similar ($P = 0.66$) between treatments. Birth date and calf BW at birth was not different ($P > 0.70$) between treatment groups.

At initiation of estrous synchronization, cows averaged 5.1 ± 0.1 BCS, 52 ± 1 d postpartum, and 6.7 ± 0.2 yr of age. Proportion cycling, mean and distribution of cow body condition, days postpartum, and age is depicted in Table 2.

Table 2. Description of cows

Item	Treatments CON	RPC
Number	70	74
Cycling, %	60.0	59.5
Non-cycling, %	40.0	40.5
Body condition, mean	5.0	5.2
Distribution, %		
< 5	40.0	21.6
5 to 6	51.4	68.9
≥ 6.0	8.6	9.5
Days postpartum, mean	51.1	52.1
Distribution, %		
≥ 70 days	11.4	14.9
50 to 70 days	40.0	43.2
< 50 days	48.6	41.9
Years of age, mean	6.7	6.6
Distribution, %		
3 to 4	20.0	21.6
5 to 9	65.7	68.9
> 10	14.3	9.5

Proportion of cows that were estrual (59%) at initiation of estrous synchronization was similar ($P = 0.82$) between supplementation treatments (Table 2). Conception to fixed-time AI tended ($P = 0.13$) to be greater for RPC (58.1%) than CON (45.7%; Table 3). Proportion of non-cycling and cycling cows conceiving to fixed-time AI was not different ($P > 0.20$) between treatments but was numerically greater for RPC compared to CON. Proportion of young cows (3 to 4 yr of age) conceiving to fixed-time AI was greater ($P = 0.06$) for RPC compared to control (Table 3). In contrast, proportion of mature cows (≥ 5 yr of age) conceiving to fixed-time AI did not differ ($P = 0.46$) between prepartum supplementation groups. Final pregnancy rate was similar ($P = 0.46$) between CON and RPC, averaging 90.8%.

Table 3. Conception rates

	Treatments	
Item, % (n)	CON	RPC
Pregnancy rate to AI	45.7 (32/70)	58.1 (43/74)
Cycling cows	45.2 (19/42)	58.1 (25/43)
Non-cycling cows	46.4 (13/28)	58.1 (18/31)
Cow age		
3 to 4 yr	35.7 (5/14)	68.8 (11/16)
≥ 5 yr	48.2 (27/56)	55.3 (32/58)
Final pregnancy rate	92.8 (64/69)	89.2 (66/74)

Discussion

Similar to our study, supplementation of prepartum dairy cows for 25 d prepartum with 60 g/hd/d RPC (15 g dietary choline) had no effect on BW or DMI (Janovick Guretzky et al., 2006). Zahra and coworkers (2006) also reported that 56 g/hd/d RPC (14 g dietary choline) supplementation of dairy cows had no effect on prepartum DMI. Interestingly, these researchers observed that DMI of thin cows (BCS < 4) was not altered by RPC supplementation but that DMI of fat cows (BCS ≥ 4) receiving RPC was greater than that of fat control cows. In contrast to our study, Jaeger et al. (2008) reported that RPC supplementation for 50 d prior to calving was associated with greater ADG during the supplementation period compared to control cows; however, these researchers also indicated that prepartum RPC-supplementation of beef cows had no effect on BCS, BF, MB, or LMD.

Increased plasma NEFA results in increased uptake by the liver where NEFA are esterified to triglycerides, oxidized to ketone bodies, or oxidized to carbon dioxide. The esterification of NEFA to triglycerides and their export as VLDL involves choline. In addition, choline serves as a methyl donor for the synthesis of carnitine. Carnitine is essential for fatty acid oxidation. Decreased plasma NEFA of RPC-supplemented cows reported by Pinotti and coworkers (2003) may have resulted from more efficient liver function and improved lipid metabolism. Improved utilization of NEFA and increased synthesis of carnitine could explain why a greater proportion of RPC-supplemented cows, and especially young cows, in our study tended to conceive to fixed-time AI. In contrast, dairy cows receiving either 0, 15, 30 or 45 g/d dietary choline from wk 5 postpartum to wk 21 postpartum required more services per cow and were open more days as choline intake increased (Erdman and Sharma, 1991). Conversely, these authors also reported that increased milk yield was associated with RPC-supplementation and speculated that reduced reproductive performance was more related to increased milk production than to the effect of RPC. Supplementation of RPC during only the prepartum period in the current study presumably eliminates potential negative effects on reproduction.

Implications

Supplementation of prepartum beef cows with rumen-protected choline did not affect daily gain or body composition during the supplementation period. However, choline-supplemented cows tended to conceive to fixed-time artificial insemination in greater numbers than control cows. In addition, a greater proportion of 3 and 4 yr old cows conceived to fixed-time AI if they received rumen-protected choline during the later portion of the third trimester. Although, the mechanisms responsible for improved reproductive response are not understood, these data were interpreted to suggest that choline supplementation during the prepartum period may improve subsequent reproductive performance, especially in young beef cows. Further investigation appears warranted.

Acknowledgements

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REPRODUCTIVE PERFORMANCE OF BISON AT THE NATIONAL BISON RANGE

M. J. Borgreen[1], T. J. Roffe[1], K. Phillips[2], and J. G. Berardinelli[2]
[1]U. S. Fish and Wildlife Service, Bozeman, MT 59718
[2]Montana State University, Bozeman 59717

ABSTRACT: The recruitment of calves at the National Bison Range (NBR) near Moiese, MT has dropped from the historic average of 87 to 33 calves per 100 breeding age cows in 2008. The purpose of this study is to monitor the NBR bison pregnancy rates and calf recruitment in an effort to determine where in the reproductive cycle NBR female bison fail to recruit calves. The reproductive cycle was divided into 3 stages: conception to early embryonic development; maintenance of pregnancy during the second and third trimesters; and, calving to recruitment. Herein we report results related to the first two stages. Pregnancy rate of 41 cows, ages 4 to 12 yr, were determined by transrectal ultrasonography of the uterine contents of each cow in early October at the NBR's annual roundup. Pregnant cows were given a number, painted on the right flank of each animal, using a commercial hair-bleaching agent. Numbers were used to identify individual cows throughout the study period. A blood sample, collected by jugular venepuncture and fecal sample were collected from each cow and from 3 bulls for assay of progesterone (P4) by RIA. Serum and fecal P4 concentrations from anovular cows and bulls were used to determine a P4 baseline to evaluate pregnancy rate using P4 concentrations in fecal samples collected from cows, collected in the field, in early January. The criterion used for evaluating pregnancy was based on fecal P4 concentrations of bulls and anovular cows plus 2 SDs or 20.4 ng/g of feces. Additional fecal samples were collected from cows that were not painted with a number to better estimate the overall pregnancy rate of the herd. During the 2008 roundup, 28 of 41 (68%) cows were determined to be pregnant. During the second trimester samples were collected from 26 of the 28 numbered cows and 28 unmarked cows. Of those, 15 (58%) numbered and 4 (14%) unmarked cows had fecal progesterone concentrations that exceeded the criterion for a P4 concentration considered to be consistent with pregnancy. By establishing the timing of reproductive failures, managers will be able to focus their efforts in determining the causative agent(s) of the reproductive failure in this herd.

Key words: bison, fecal progesterone, pregnancy rate

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Introduction

The 18,500 acre National Bison Range (NBR) near Moiese, MT was established by Congress in 1908. In 1909-1910, 40 bison were brought to the NBR, and by the early 1920's the herd size was near 300 animals. NBR bison have historically had little problem with recruitment, which for the purpose of this study is defined as the survival of a calf until the bison roundup that occurs each year usually during the first week of October. The National Bison Range Fenced Animal Management Plan states that over 32 years (1956-1987) the average recruitment was 87%. The lowest recruitment recorded during that same period of time was 72% in 1970. Then bison of the NBR have had little trouble with diseases that are known to affect reproduction. According to the National Bison Range Fenced Animal Management Plan, the herd was certified brucellosis free in 1983 by the Montana Department of Livestock. In 1979 it was believed that the bison experienced an outbreak of Leptospirosis characterized at the time by late calving. In 1980 calf recruitment had dropped to 74%. Personnel at the NBR initiated an annual Leptospirosis vaccination program for an unknown length of time and the problem seemed to be resolved, as calf recruitment was back up to 85% in 1981. During the last 3 years (2005-2007) recruitment has dropped to an average of 54%, and the cause of this decrease in production is unknown (U.S. Fish and Wildlife Service, unpublished data). The objective of this study was to determine when during the reproductive cycle of bison at the NBR are failing to recruit calves. Specifically we evaluated losses associated with early embryonic and fetal loss by transrectal ultrasonographic evaluation of the contents of the uteri of female bison and by fecal concentration of progesterone during gestation.

Materials and Methods

This study will be conducted over a two-yr period. For the purpose of this study in each year gestation of female bison will be broken into 3 periods. In the first period we will monitor conception to early embryonic development. In early October, pregnancy rates will be determined during the NBR annual bison roundup using transrectal ultrasonographic evaluation of the contents of the uteri of cows with a Titan Ultrasound Imaging System (SonoSite, Bothell, WA) equipped with a selectable 5 to 10 MHz transducer. The accuracy of ultrasonic evaluation is approximately 100% at detecting

pregnancy between 21 and 30 d after fertilization. Given the detection limit of 21 to 30 d there is the possibility of failing to detect the presence of an embryo in those females that bred late in the breeding season. Twenty-eight cows were determined to be pregnant at the 2008 roundup. These cows received a number on the right flank using a commercial hair-bleaching agent that allows us to identify these individuals for collection of fecal samples throughout the remainder of gestation.

Blood and fecal samples were collected from 41 cows (4 and 12 yr of age) at roundup. These samples were used to validate assays for fecal progesterone concentrations. Fecal progesterone concentrations were correlated with serum concentrations of progesterone to evaluate the accuracy of fecal progesterone concentrations for determining a criterion for the minimum fecal progesterone concentration associated with pregnancy.

During the second period cows will be monitored for maintenance of pregnancy using fecal progesterone concentrations. Limiting the invasiveness of sample collection is an important consideration when selecting a sampling method in bison. Pregnancy determination using fecal steroid concentration has been validated using bovine (Desaulniers, et al. 1989), and bison feces (Kirkpatrick et al., 1992). Using fecal samples to determine pregnancy status will allow us to avoid the unnecessary handling of cows and will limit animal stress during pregnancy. Fecal material has been collected from adult cows and assayed for progesterone using solid phase radioimmunoassay as described in Kirkpatrick et al. (1992), and Custer et al. (1990). Samples were collected in January (second trimester) and in March (third trimester). Extraction of progesterone from fecal samples of bison was performed by the method described by Brown et al. (2005).

The method for collecting fecal samples in the field involved personnel that found and monitored groups of bison that included cows. Personnel observed these cows until a cow defecated. Any cow that defecated in that group was then marked with livestock paint using a paint dart. Then, the group was slowly pushed from that area so that fecal samples from cows could be safely collected. Every effort was made to collect as many samples from bleach-numbered cows as possible.

In the third period, calving to recruitment will be monitored by two methods. First, monthly calf counts will be conducted from late March until mid September. At least 200 bison will be observed and classified as calves or non-calves and the observed ratio will be extrapolated to the whole herd. Also, fecal samples from cows will be collected for progesterone assays during each monthly calf survey or until 2 consecutive fecal progesterone concentrations are below the minimum criterion established for progesterone concentrations in pregnant cows. These two rates should provide a reliable estimate of fetal losses that can be used to compare pregnancy rates estimated earlier in gestation. Bleach-numbered cows will be counted and monitored until it can be determined a cow is tending a calf. The calf will be assumed to have been lost if it is not present with the cow in subsequent surveys.

Results

In 2008, calf recruitment of the NBR was at an all time low of 33% (Figure 1). During the 2008 roundup 41 breeding-age cows were examined. Of those, 28 (68%) were determined to be pregnant via ultrasonic evaluation (Table 1).

Bull and anovular cow feces were used to determine extraction efficiencies and establish a pregnancy cutoff. Extraction efficiencies were approximately 79%. The minimum fecal concentration of progesterone for evaluating pregnancy was determined from concentrations from fecal samples collected from bulls and anovular cows. The mean concentration of these samples was to be 20.4 ng/g of feces. This was based on the concentration that was 2 SD above the average of bulls and anovular cows fecal progesterone concentrations.

During the second trimester, all 28 numbered cows were observed alive, and samples were collected from 26. Additionally, 28 samples from unmarked cows were collected. Of the numbered cows, 15 of 26 (58%) and 4 of 28 (14%) unmarked cows had fecal concentrations of progesterone that exceeded the criterion for pregnancy.

Fecal samples from numbered cows collected in January were extracted and assayed a second time to determine the repeatability of the assay. Results from the second assay were considerably different than the first assay. Of the 26 numbered cows 24 (90%) of them had fecal concentrations of progesterone that exceeded our criterion for pregnancy. Likewise of the 28 unmarked females 15 (58%) exceeded the criterion for concentrations of progesterone consistent with pregnancy. In order to evaluate the repeatability and reliability of our extraction and assay procedures, a subset was then homogenized and assayed a third time. The reason for this was that progesterone may be stratified within the fecal samples. So that taking an aliquot for assay from the top of our fecal sample may be different than taking an aliquot from the middle or bottom of our sample. Results from this assay were both higher and lower than the original assays (Table 2).

Discussion

The list of possibilities that cause decreases in recruitment is lengthy. The bison could be experiencing an outbreak of a viral disease, a mineral or vitamin deficiency, an increase in predation or the herd is over habitat capacity. The magnitude of the list of diagnostics makes it essential that the timing of the recruitment failure be established before further testing is done to determine the exact cause of the failure.

The variation observed in fecal P4 in the same pregnant females among samples collected at roundup, in

January, and homogenized samples from January's collection indicate that fecal progesterone concentrations do not accurately or repeatability assess the pregnancy status of a cow. Additional assays of homogenized fecal samples are necessary to determine the repeatability and usefulness of fecal P4 assays as a non-invasive pregnancy detection method.

Implications

The results from this study will be the foundation for future research into the exact cause of the decrease in recruitment. Data from this project will help managers determine if a change in management is warranted. If no reduction in recruitment is detected the problem has either resolved itself and no further research is needed or adjustments to our sampling or analyses are warranted. Data will reinforce existing data on collection and extraction techniques for fecal progesterone concentration as a criterion for determining pregnancy status of a herd of bison.

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Table 1. Number of pregnant and non-pregnant cows determined by ultrasonic evaluation during the 2008 roundup in early October.

Pregnant	Non-Pregnant	Total
28	13	41

Table 2. Progesterone concentrations (ng/g of feces) for 10 cows. Assay 1 and Assay 2 aliquots were collected from the sample before homogenization, while Assay 3 was collected after.

Assay 1	Assay 2	Assay 3
23.8	20.7	17.5
27.1	84.5	91.1
22.2	24.8	16.1
60.1	156.9	52.3
11.7	50.9	29.5
18.3	147.7	40.2
5.8	24.5	13.8
5.8	14.5	9.6
17.8	101.9	33.7
4.7	10.3	10.0

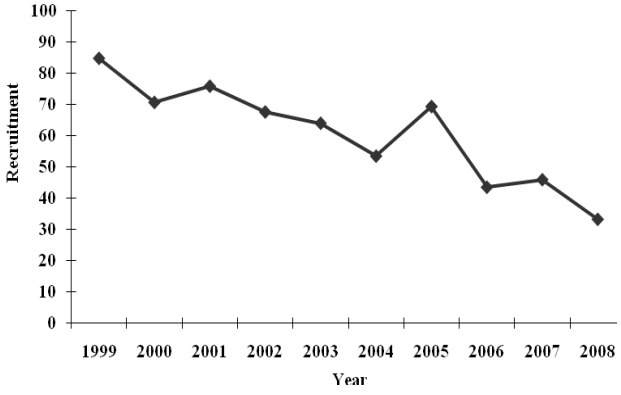

Figure 1. Calf recruitment at roundup for the National Bison Range bison herd over the last 10 yr. The long-term average recruitment is 87 calves per 100 breeding age cows. Calf recruitment has steadily declined over the last 10 years to an all time low of 33% in 2008.

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DURATION OF DAILY BULL EXPOSURE ON LEPTIN CONCENTRATIONS DURING RESUMPTION OF OVULATORY ACTIVITY IN PRIMIPAROUS, POSTPARTUM, ANESTROUS, BEEF COWS[1]

J. R. Olsen[2], S. A. Tauck[2], J. R. C. Wilkinson[2], D. H. Keisler[3], J. G. Berardinelli[2]
Montana State University, Bozeman, MT, USA[2], University of Missouri, Columbia, MO, USA[3]

Abstract: The objective of this experiment was to determine if duration of daily bull exposure alters leptin concentrations in primiparous, anovular, suckled, beef cows. The null hypotheses were that leptin concentrations and concentration patterns do not differ among cows exposed to bulls for 0, 6, or 12 h daily, and that there is no relationship between the resumption of ovulatory activity (OA) and leptin concentrations in cows exposed to bulls for 0, 6, or 12-h daily. At 51.5 ± 2.3 d (± SE) after calving, cows were assigned randomly to be exposed for 12 (BE12; n = 15) or 6 h daily (BE6; n = 14) to mature bulls, or not exposed to bulls (NE; n = 10) for 45 d. The cow to bull ratios for BE12 and BE6 cows were 7.5:1 and 7:1, respectively. Blood samples were collected from each cow by jugular venepuncture at 2-d intervals from start of experiment (D 0) and assayed for progesterone and leptin concentrations. Likewise, ovaries of each cow were examined ultrasonographically for the presence of a corpus luteum. There was a treatment by day interaction ($P < 0.05$) for leptin concentrations, indicating that leptin concentration patterns differed among treatments. This interaction was best explained the by the relationship between mean leptin concentrations and proportions of BE12, BE6, and NE cows that resumed OA. Leptin concentrations tended to be greater ($P = 0.09$) in cows that resumed OA than cows that did not resume OA. There was a tendency for a linear ($P = 0.09$) relationship between mean leptin concentrations and hours of bull exposure. These results indicate that bull exposure affects temporal patterns of leptin concentrations. However, this effect may be indirectly mediated through the physiological mechanism that stimulates the resumption of ovulatory activity in primiparous, postpartum, anestrous, beef cows.

Key words: bull biostimulation, leptin, postpartum anestrus, cows

Introduction

The biostimulatory effect of bulls involves interactions between bulls and cows by which bulls stimulates the resumption of ovulatory activity (OA) in postpartum, primiparous, anovular, suckled beef cows (Custer et al., 1990). Bull exposure is known to alter cortisol concentrations and the characteristics of their pulsatile patterns (Tauck et al. 2007). Furthermore, there is the possibility that the biostimulatory effect of bulls to accelerate resumption of ovulatory activity involves metabolic changes in cows that are induced by changes in cortisol concentrations; specifically changes in adipose metabolism (Landaeta-Hernandes et al., 2004, Wilkinson et al., 2007). Stumpf et al. (1992) reported that cows of low body condition have a greater reduction in postpartum interval when exposed to bulls than cows of high body condition. Recent research indicates possible physiological mechanisms by which energy balance modulates reproductive functions. Leptin, an adipocyte-derived hormone is an important factor for the control of energy balance, satiety, and food intake. Also, leptin may modulate the hypothalamic-hypophyseal-gonadal axis at many levels (Tene-Sempere, 2007). The objective of this experiment was to determine if duration of daily bull exposure alters leptin concentrations in primiparous, anovular, suckled, beef cows. The null hypotheses were that leptin concentrations and concentration patterns do not differ among cows exposed to bulls for 0, 6, or 12 h daily, and that there is no relationship between the resumption of OA and leptin concentrations in cows exposed to bulls for 0, 6, or 12 h daily.

Materials and Methods

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

Animals and Treatments. Thirty-nine, spring-calving, two-yr-old Angus X Hereford primiparous, suckled beef cows and four mature epididymectomized Angus X Hereford bulls were used in this experiment. Cows and calves were maintained in a single pasture and had no contact with bulls or their excretory products from the previous breeding season until the start of the experiment (D 0). Average calving date for these cows was Feb. 18, 2008. Before the start of the experiment, cycling status of each cow was rated by two ultrasound examinations of each ovary for the presence or absence of a corpus luteum. The first and second ultrasonic examinations were conducted 10 and 2 d, respectively, before the start of the experiment. Cows that did not

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exhibit the presence of a corpus luteum on either ovary in both ultrasound examinations were used in this experiment.

The interval from calving to D 0 averaged 51.5 ± 2.3 d. Two d before the start of the experiment cows were stratified by calving date, calf birth weight, dystocia score, cow body weight, cow BCS, and sex of calf and assigned randomly to be exposed to bulls for 12 h daily (BE12; n = 15), 6 h daily (BE6; n =14) or not exposed to bulls (NE; n = 10) for 45 d.

Facilities and Daily Bull Exposure. Cows were housed within pens in separate lot areas. Pens within the south lot were used to maintain BE12 and BE6 cows while pens within the north lot were used to maintain NE cows. A common holding pen, approximately 0.35 km from the lot that housed NE cows and 30 m from the lot that housed BE12 and BE6 cows, was used to house bulls before and after daily exposure periods. During daily exposure periods two bulls were moved from the common holding pen into the pen that housed BE12 cows and two bulls were moved into the pen that housed BE6 cows. Cows in each treatment were exposed to bulls at 0700 h each day for 45 d (D 0 to 44). At 1900 and1300 h bulls were removed from pens that housed BE12 and BE6 cows, respectively, and housed in the common holding pen until the following day. Cows in each treatment could not see or smell bulls before or after daily exposure periods. Figure 1 illustrates pen arrangements and method of daily bull exposure.

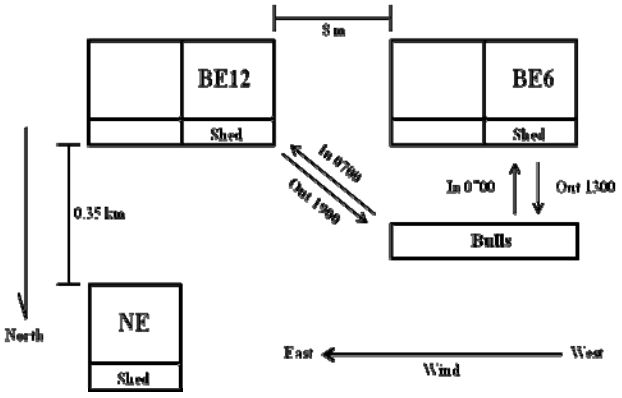

Figure 1. Facilities where cows were housed during experiment and schedule of daily bull exposure.

Nutrition. Cows had free access to good quality, chopped mixed-grass alfalfa hay, and any pasture grasses that were available before the start of the experiment. Once cows and calves were moved into pens they were given free access to the same hay, 0.5 kg•hd-1•d-1 cracked corn, water, and a trace mineral-salt supplement. Average body weight of cows was 467.5 ± 37.7 kg. The TDN of the diet was 110% of the recommended energy requirement for lactating beef cows with a mature weight of 533 kg (NRC, 1996). Bulls were fed the same diet as cows.

Blood Sampling, Leptin and Progesterone Concentrations, and Resumption of Ovulatory Activity. Blood samples were collected from each cow by jugular venepuncture every other day from D 0 to the end of the experiment (D 44). Leptin concentrations were determined in triplicate and quantified by using a competitive liquid-liquid phase, double-antibody leptin RIA procedure described previously (Delavaud et al., 2000). The intra and inter assay coefficients of variation were 2.01% and 3.22%, respectively . Serum was assayed for progesterone concentration in duplicate using solid-phase RIA kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Intra- and interassay CV for a serum pool that contained 2.2 ng/mL of progesterone were 10.2 and 15.4%, respectively, and 8.9 and 11.8%, respectively, for a pool that contained 5.75 ng/mL. Progesterone concentrations in these samples were used to determine the interval from calving to resumption of OA, interval from the start of the experiment to resumption of OA, and the proportion of cows that resumed OA during the experiment. An increase of progesterone concentration, above the average progesterone baseline of individual cows in three consecutive samples that exceeded 1 ng/mL was used to determine the occurrence of resumption of OA. Intervals from calving and D 0 to resumption of OA were determined by the number of days from the treatment to the lowest inflection point before a rise in three consecutive samples that exceeded 1 ng/mL. Progesterone concentrations and resumption of OA were confirmed by transrectal ultrasonographic examination of ovaries of each cow using a Titan ultrasound with a 7.5 to 10 MHz rectal transducer every other day throughout the 45-d exposure period used in this experiment (SonoSite Inc., Bothell, WA, USA). The presence of a corpus luteum in the same anatomical position of an ovary in 4 successive scans was used as evidence to confirm resumption of OA. Cows that failed to exhibit a rise in progesterone and did not have a corpus luteum in their ovaries were assigned an interval from calving or the start of treatment to the end of the experiment.

Statistical Analyses. Intervals from D 0 to resumption of OA were evaluated by ANOVA for a completely randomized design using PROC GLM of SAS (SAS, Cary, NC). The model included treatment (TRT) and means were separated using Bonferroni Multiple Comparison tests. Linear regression analyses were used to determine the relationship between intensity of bull exposure (hours/d) and intervals (days) from calving and the start of the experiment to resumption of OA using the PROC REG procedure of SAS. Data for intervals from calving and from D 0 to resumption of OA showed heterogeneous variances among treatments using Bartlett's Box F-test. Therefore, data from the start of the experiment to resumption of OA that were used in ANOVA were transformed by raising intervals to the power of 10.3. Least squares means and standard errors of means for intervals and D 0 to resumption of OA, reported herein, were transformed to original values after

analysis. Differences in proportions of cows that resumed OA during the experiment were analyzed by chi-square using the PROC FREQ procedure of SAS.

Leptin concentrations were analyzed by PROC MIXED repeated measures analysis of SAS. The model included TRT, Animal as experimental unit, Day as the repeated measure, and the TRT by Day interaction and means were separated using Bonferroni Multiple Comparison tests. Linear regression analyses between leptin concentrations and hours of daily bull exposure were conducted using PROC REG procedure of SAS.

Results

Interval from D 0 to resumption of OA was shorter ($P < 0.05$) for BE12 cows than for NE cows. However, interval from D 0 to resumption of OA did not differ ($P > 0.10$) between BE6 cows and BE12 or NE cows. Although, the proportion of BE12 and BE6 cows that resumed OA during the experiment did not differ ($P > 0.10$), the proportions of cows that resumed OA for both BE6 cows and BE12 cows were greater than for NE cows (Table 1).

There was a treatment by day interaction ($P < 0.05$) for leptin concentrations. The data indicated that the temporal patterns of leptin concentrations differed among NE, BE6, and BE12 cows. However, leptin concentrations tended to be greater ($P = 0.09$) in cows that resumed OA than cows that did not resume OA, indicating this interaction may be due to cycling status. In order to better understand this treatment by day interaction, the experiment period was grouped into four intervals; D 0 to 10, D 11 to 20, D 21 to 30, and D 31 to 40.

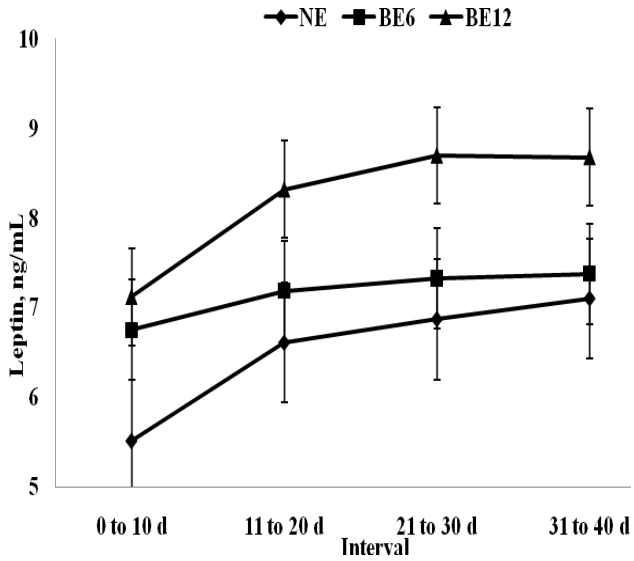

Figure 2. Least squares means of leptin concentrations for 10-d intervals in cows exposed to bulls for 0 (NE), 6 (BE6), and 12 (BE12) h daily. Bars associated with points represent standard errors for individual means. Interval by treatment interaction; $P < 0.05$.

Figure 2 illustrates patterns of leptin concentrations by treatment, wherein leptin exhibits a similar pattern in all levels of daily bull exposure. However, it seems that as the duration of bull exposure increases, treatments exhibit leptin patterns at slightly higher levels, respectively. Leptin concentrations seem to increase through the first 20 d of the experiment and then level out. Furthermore, leptin concentrations from D 21 to 30 for BE12 cows tend to be greater ($P = 0.10$) than NE cows. Again, there is the possibility that this could be attributed to the proportion cycling within each treatment. However, when samples taken after animals had resumed OA were removed from the analysis, there was a similar significant treatment by day interaction ($P < 0.05$) as well (Figure 3).

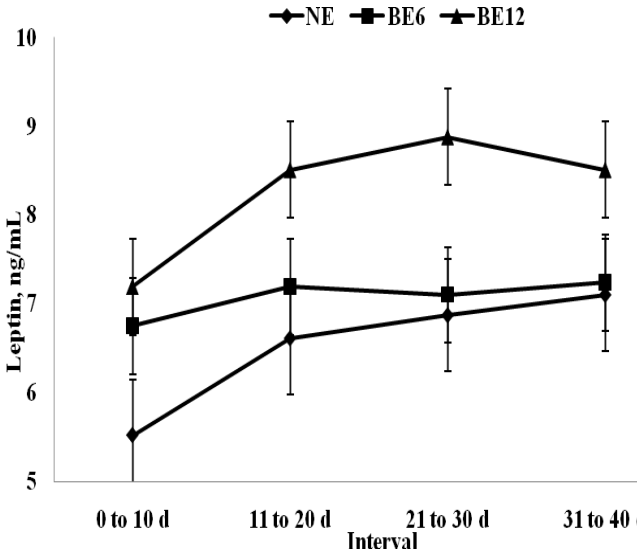

Figure 3. Least squares means of leptin concentrations for 10-d intervals in cows exposed to bulls for 0 (NE), 6 (BE6), and 12 (BE12) h daily and before the resumption of ovulatory activity. Bars associated with points represent standard errors for individual means. Interval by treatment interaction; $P < 0.05$.

Similarly, concentrations of leptin increased faster within the first 20 d than through the rest of the experiment. However, in this analysis of samples from cows before they resumed OA, leptin concentrations from D 21to 30 were greater ($P < 0.05$) in BE12 than both BE6 and NE cows. However, there was a tendency for leptin concentrations to be greater ($P = 0.09$) in cows that had resumed OA during the experiment than cows that did not resume OA, 8.0 and 6.9 ng/mL, respectively. There was a tendency for a linear relationship (b1 = 0.123 ng/h; $P = 0.09$) between mean leptin concentrations and hours of bull exposure.

Discussion

The purpose of this study was to evaluate changes in leptin concentrations in primiparous, postpartum, anovular, suckled, beef cows that were exposed to bulls for durations of 0, 6, and 12 h daily.

Even though, mean leptin concentrations did not differ among treatments, we found that there was a treatment by day interaction for leptin concentrations. Interestingly, across all treatments, leptin concentrations tended to be greater in cows that had resumed ovulatory activity during the experimental period (D 0 to D 44) than in cows that had not resumed ovulatory activity (OA) by the end of the experiment. Specifically, cows that had resumed OA during the experimental period had mean leptin concentration of 8.0 ng/mL, whereas, cows that did not resume OA by the end of the experiment (D 44) had a mean leptin concentration of 6.9 ng/mL. This suggests that the treatment by day interaction for leptin concentrations perhaps is more directly related to cycling status of cows and indirectly influenced by the biostimulatory effect of bulls to hasten the resumption of OA in the postpartum, anovular cow.

If cycling status is more responsible for temporal changes in leptin concentrations, one could expect that the proportion of cows cycling would correlate with mean leptin concentrations. However, we found a tendency for a positive linear relationship between h of bull exposure (0, 6, and 12 h daily) and mean leptin concentrations, wherein the proportion of cows exposed to bulls for 6 and 12 h daily did not differ, but were significantly greater than that of cows not exposed to bulls. Though it is only a tendency, this linear relationship suggests the possibility that the biostimulatory effect of bulls may have a direct influence on temporal patterns of leptin concentrations.

As duration of daily bull exposure increased it appeared that temporal leptin concentrations increased. Again, there is the possibility that this could be attributed to the proportion of cows that resumed OA within each treatment. However, removing those samples collected after cows had resumed OA, there was a similar if not more significant treatment by day interaction. These interactions indicate that bull exposure can influence leptin concentrations and this idea is further supported by the tendency for a linear relationship between mean leptin concentrations and duration of daily bull exposure. Interestingly, cows exposed to bulls for 12 h daily had a mean interval from D 0 to resumption of OA of 30.6 d, that corresponded to the interval (D 21 to 30) during which leptin concentrations in these cows showed the greatest difference from cows not exposed to bulls.

Wilkinson et al. (2007) reported that non-esterified fatty acids (NEFA) concentrations were significantly lower in cows that were exposed to bulls than cows not exposed. If leptin concentrations are high, this would be a signal of satiety to the brain and would be concurrent with a decrease in the mobilization of NEFA from adipose tissue (Tene-Sempere, 2007). These results are complementary to those observed in the present study.

In conclusion, daily bull exposure reduced the interval to resumption of OA and alterd the temporal pattern of leptin concentrations in the postpartum, anovular, suckled, beef cows. This suggests that the biostimulatory effect of bulls may have a metabolic component.

Implications

The results of this study suggest that leptin concentrations are increased by both the biostimulatory effect of bulls and the resumption of ovulatory activity in postpartum, suckled cows. Further investigations are necessary to explain how the presences of bulls influence leptin; and to understand the role of changes in leptin concentration patterns during the resumption of ovulatory activity in the postpartum, primiparous, anestrous, suckled, beef cow.

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Table 1. Number of cows per treatment and least squares means for intervals from calving to resumption of ovulatory activity (ROA) and proportions of cows that ROA during the experiment for primiparous, anestrous, suckled, beef cows exposed to bulls for 12-h daily (BE12), 6-h daily (BE6), or not exposed to bulls (NE) for 45 d starting 51.5 ± 2.3 d (± SE) after calving

Variable	Treatment [a]			SEM	*P* -Value
	BE12	BE6	NE		
n	15	14	10		
Interval from D0 to ROA, d[b]	30.6^{x}	35.0^{x,y}	43.0^{y}	10.6	<0.05
Proportion that ROA, %	60.0^{x}	64.3^{x}	10.0^{y}	8.0[c]	<0.05

[a]Means and proportions that lack common superscript differ ($P < 0.05$).
[b]Cows that failed to ROA were assigned an interval from calving to the end of the experiment.
[c]X^2 value.

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INVESTIGATION OF A NOVEL NON-SURGICAL METHOD OF ARTIFICIAL INSEMINATION FOR SHEEP

P.H. Purdy*[1], H.D. Blackburn[1], B. Larson[2], R. Stobart[2]; USDA-ARS-NCGRP National Animal Germplasm Program, Fort Collins, CO USA[1], University of Wyoming Department of Animal Science, Laramie, WY USA[2]

ABSTRACT: Transcervical artificial insemination (**AI**) with sheep is not frequently used in the US due to low fertility rates. Consequently, laparoscopic AI has been employed to circumvent this situation. The problem with this technique is that while it provides satisfactory levels of fertility the degree of technical expertise necessary to perform the procedure, the costs associated with purchasing equipment, and/or the costs of hiring an inseminator are prohibitive for many producers. Therefore, the purpose of this study was to investigate a transcervical method of AI that is nonsurgical, simple to perform, and inexpensive. The estrous cycles of 40 ewes were synchronized using CIDRs for 12 days. Upon CIDR removal vasectomized rams (n = 4) fitted with raddle harnesses were turned out with the ewes (one ram per 10 ewes in separate pens) to detect estrus. After ewes were observed in estrus they were removed from the pen and inseminated twice with either fresh (n = 19 ewes) or frozen-thawed semen (n = 21 ewes) 15 and 24 hours after observed estrus using a spiral sow insemination catheter. Least square means for lambing rates for fresh and frozen-thawed treatments were 55% and 9%, respectively ($P < 0.05$). However, mean prolificacy rates did not differ ($P > 0.05$) for fresh (1.3) or frozen-thawed (1.0) treatments. These results demonstrate that this economical, easy to use method of non-surgical AI for sheep is feasible but the results indicate that improvement is needed. Consequently, future studies will be performed with the goal of improving fertility with this technique, particularly when utilizing frozen-thawed semen, which should prove valuable to sheep producers.

Key Words: Artificial insemination, Sheep, Semen.

Introduction

Ram semen can be readily cryopreserved, stored indefinitely, and still produce acceptable levels of fertility (> 50%) provided that laparoscopic insemination is used (Wulster-Radcliffe et al., 2004; King et al., 2004; Fair et al., 2005). When cervical insemination is used the results are more variable and dramatically different with reports of lambing rates from 4 to 75% (Wulster-Radcliffe et al., 2004; King et al., 2004; Fair et al., 2005; Paulenz et al., 2005). Still, non-surgical AI is highly desirable because of its low cost and ease of use.

AI using cryopreserved semen is advantageous to producers because it enables the use of diverse genetics and increases genetic progress. Currently AI is not readily used in the US because of a lack of available frozen ram semen and the difficulty and expense of insemination. The most effective sheep AI method is the laparoscopic (surgical) procedure which is expensive and requires a trained technician. Cervical and vaginal insemination is inexpensive but fertility is generally low because the cervix is not readily breached and as a result the semen is rarely deposited in the uterus (King et al., 2004; Fair et al., 2005; Paulenz et al.,2005). Therefore, a method is needed to enable a producer to non-surgically inseminate sheep.

The goal of this research was to explore a new non-surgical method of sheep AI that is inexpensive, easy to perform and could be conducted by producers.

Materials and Methods

Animal Care. All procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee. Two post-pubertal Rambouillet rams and 40 western white-faced ewes were fed a diet that met all of their nutritional needs and ad libitum water.

Semen Processing. Semen was collected from rams using electroejaculation (Evans and Maxwell, 1987). An aliquot of each ejaculate was diluted in 37ºC Tris buffered saline (Purdy and Graham, 2004) and evaluated using bright field microscopy to ensure that all samples used for fresh or frozen-thawed insemination had an initial minimum of 70% total sperm motility.

Pooled ejaculates from two rams were used for fresh insemination and were diluted in 37ºC Tris buffered saline to 400 x 10^6 sperm/mL and loaded into 0.5 mL French straws (IMV USA, Minneapolis, MN). The samples were maintained at 23ºC until insemination, which was always less than 2 h, and the motility was periodically evaluated to ensure the minimal quality (70% motility).

Ejaculates from the same two rams were used for frozen-thawed insemination. Ejaculates were collected and diluted with Tris-egg yolk-glycerol media (15% egg yolk, 5% glycerol by volume; Sanchez-Partida et al., 1998) to 400 x 10^6 sperm/mL in one step. The samples were then cooled to 5ºC over two hours, held at this temperature for 24 hours, loaded into 0.5 mL French straws, and frozen using a programmable freezer and the following freeze curve: 5ºC to -5ºC at 4ºC/min, -5ºC to -110ºC at 25ºC/min, -110ºC to -140ºC at 35ºC/min. The samples were then plunged into the liquid nitrogen for storage. Samples were thawed by placing the straws in a 37ºC water bath for 30s. Frozen-thawed samples were analyzed for motility using computer automated semen analysis (Hamilton-Thorne Motility Analyzer, Beverly, MA) as described by Purdy (2006).

Estrous Synchronization and Heat Detection. Cycling ewes aged three to seven years were treated with CIDRs for 12 days and assigned to treatment groups which

were stratified across the available ewes based on semen treatment (fresh or frozen-thawed) and ewe age. Once the CIDRs were removed the ewes were placed in pens (10 ewes per pen) and a vasectomized ram fitted with a raddle harness and crayon was introduced into each pen. Ewes were considered to be in estrus when they would stand and allow the vasectomized ram to mount. The time of mounting was noted and the marked ewes were removed from the pen. The ewes were exposed to different rams every two hours.

Artificial Insemination. Ewes were inseminated 15 and 24 hours after observed estrus using a single straw of semen containing 200 x 10^6 sperm at each insemination time. Straws were loaded into an All-2-Mate goat insemination gun, covered with an Apex sheath (Figure 1-A), and then inserted into a spiral tip swine insemination catheter (Figure 1-B; Continental Plastics, Delavan, WI). The catheter was cut prior to loading so that the prepared AI gun could be pushed forward and the tip of the gun would protrude from the opening in the spiral catheter (Figure 1-C). The semen could then be expelled from the gun into the ewe with minimal back-flow into the catheter. Ewes were placed in a squeeze chute and the vagina of the ewe was opened using a 4 inch duck billed speculum. The tip of the spiral catheter was lubricated using a non-spermicidal obstetric lubricant and inserted into the cervix of the ewe. The catheter was threaded through the cervix of the ewes using a slight forward pressure and the semen was deposited once the catheter could be threaded no further. The catheter was then removed by reversing the threading motion. All inseminations were performed by the same inseminator. The fertility (number of ewes lambing) and prolificacy (number of lambs born per ewe lambing) was determined.

Statistics. Differences in fertility and prolificacy were determined using Chi-square analysis and included the effects of semen treatment (fresh or frozen), ewe age, and their interaction (SAS, 1985).

Results

Sperm Motility. As stated previously, all fresh samples had total motility of at least 70%. The motility of the frozen-thawed pooled samples was 32% and 19% for total and progressive motility, respectively.

Fertility and Prolificacy. The age of the ewes was a non-significant source of variation for fertility and prolificacy ($P > 0.05$). Semen treatment was a significant source of variation for fertility (55% and 9% for fresh and frozen-thawed semen, respectively). In contrast, semen treatment was not a significant source of variation for prolificacy (1.3 and 1.0 lambs were born per ewe lambing for the fresh and frozen-thawed semen treatments, respectively). The interaction of ewe age and semen treatment provided a significant source of variation (Table 1).

Discussion

The resulting fertility demonstrates two important points; first, the method was successful as live offspring were produced, and second, based on the fertility rates, modifications are needed to improve fertility with this technique, particularly with frozen-thawed semen. Significant challenges for developing this technique exist. Previous reports mentioned obstacles to achieving fertility using cervical insemination; namely the inability to traverse the ewe's cervix (Kershaw et al., 2005), the lack of inexpensive equipment for AI (Wulster-Radcliffe et a.., 2004), and varying information concerning AI dose and insemination time when using frozen-thawed semen (Salamon and Maxwell, 1995). From our research we have been able to address, to varying degrees, each of these issues.

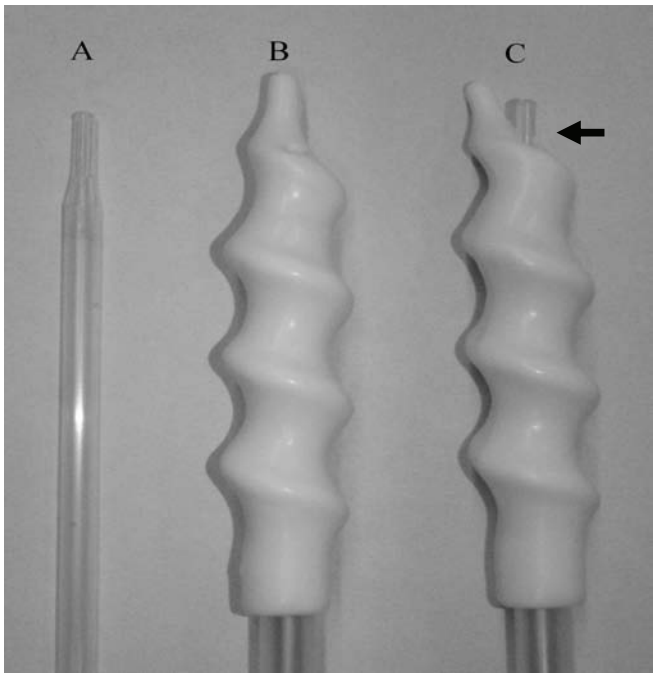

Figure 1. Illustration of the Apex sheath (A), swine spiral catheter (B), and spiral catheter fitted with the AI gun and Apex sheath ready for insemination (C). Note the arrow pointing to the Apex sheath/AI gun extended from the catheter.

Table 1. Fertility rates by ewe age and semen treatment. Treatment 1 = fresh semen and 2 = frozen-thawed semen.

Ewe age	Treatment	Pregnant/bred	% pregnant
3	1	2/2	100^a
4	1	2/5	40^{ab}
5	1	4/4	100^a
6	1	0/5	0^b
7	1	1/3	33^b
3	2	1/3	0^b
4	2	1/4	25^b
5	2	0/6	0^b
6	2	1/5	20^b
7	2	0/2	0^b

Values within a column with a different superscript differ at $P < 0.05$. SEM = 5.5.

Current hypotheses assume that it is not possible to traverse the cervix of a ewe due to its convoluted structure and even if it is possible to enter the cervix the stimulation or trauma would reduce fertility (Wulster-Radcliffe et al.,

2004). The swine catheter was used because we hypothesized its threaded structure would facilitate passage through the cervix without causing trauma. Most other cervical inseminations utilize an insemination device that had a bent tip so that it can potentially be worked into the cervix (King et al., 2004; Wulster-Radcliffe et a.., 2004; Fair et al., 2005) but we hypothesized the insertion depth is limited because the folds can not be pushed aside. In our experiences we learned that we were able to enter the cervix, and in most instances, we believe we were able to enter the uterus based on the depth of the insertion and the eventual decrease in resistance on the catheter when being threaded. This occurred for the majority of the inseminations. In the instance when it did not occur the ewes were not presenting strong signs of estrus; i.e. less engorgement of the vulva and less mucus present. When the ewes were presenting strong signs of estrus inseminations using this method typically took less than 45 seconds. We also observed that the catheters could be threaded quite easily. Only one ewe presented a trace amount of blood on the catheter when inseminated. Similarly, in a separate experiment with Black Welsh Mountain ewes, a lighter breed (~34 kg), the catheters were trimmed to decrease the outer diameter of the device and only two out of 56 ewes presented trace amounts of blood on the catheter which demonstrates the lack of invasiveness of the technique (P. Purdy, unpublished data).

In the initial adaptation of the insemination device in previous years we learned that we could deliver a semen dose although there was considerable backflow of the inseminate in many instances (P. Purdy, unpublished data). Consequently the device was modified to that which was presented here and the amount of the dose that remained in the catheter was greatly reduced. This device is cost effective on a per animal basis ($1.29 per head) when 100 animals are inseminated. This demonstrates that we have identified an inexpensive device that may be a reasonable expense for many producers.

We also believe based on our results and other reports (King et al., 2004; Fair et al., 2005) that the sperm number may need to be adjusted. The two inseminations doses contain 128 x 10^6 motile sperm which is considerable less than that reported by King et al. (2004) where 400 x 10^6 progressively motile frozen-thawed sperm were cervically inseminated and this resulted in a fertility rate of 42%.

In addition, the timing of the AI may have impacted the resulting fertility. Very little is known about the timing of AI when using cervical AI. Many reports suggest using two inseminations 12 and 24 hours post estrus detection (Salamon and Maxwell, 1995) but because we were using frozen-thawed semen we adjusted this to 15 and 24 hours post estrus detection speculating that this would be closer to the time of ovulation and thus the sperm would be in the ewe's reproductive tract for less time. As we were not using drugs to induce ovulation, in the hopes of creating an inexpensive protocol that could be used by producers, we believe that this also contributed to a decrease in fertility as the ovulation times were most likely highly variable. The significant age x semen treatment effect is interesting and could suggest that younger ewes (ages 3 to 5) are potentially better AI candidates due to greater cervical elasticity or a more consistent ovulatory pattern in relation to AI timing.

The results demonstrate that fertility can be achieved with this technique but improvements need to be made in order to reach the levels of the laparoscopic AI. While we are confident the device can successfully deliver an insemination dose more research is needed in regards to amount of sperm per AI dose and AI timing with this technique. Future efforts will focus on adjusting both of these factors with the goal of achieving greater fertility.

Implications

Many benefits can be derived from creating a user friendly technique like the one described here. First, with improvements, this technique could be used by producers to perform AI on their own sheep thereby reducing costs. This would enable a producer to purchase frozen semen from rams with desirable traits and change the genetics of their flock in a short time.

Secondly, once the technique has been improved and greater fertility achieved commercial stud services may be encouraged to collect, cryopreserve, and market genetically desirable rams. Furthermore, we previously demonstrated that ram semen could be cooled and held for up to 48 hours at 5ºC prior to cryopreservation and this did not impact fertility when laparoscopic inseminations were performed indicating the samples were able to maintain their fertilizing ability (Purdy, 2006). Therefore, this approach could also facilitate the sale and use of fresh semen collected at various studs and sent to producers, much the same way the swine industry utilizes highly productive genetics. Thus, this could benefit both the producers, by enabling them to purchase cataloged semen in a manner similar to bull semen, and the commercial enterprises because it would expand their stud service operations.

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EFFECT OF SEX OF CO-TWIN AND BREED ON EWE FLOCK PRODUCTIVITY

V.A. Uthlaut*, G.E. Moss, B.A. Larson, B.M. Alexander
Department of Animal Science, University of Wyoming, Laramie

ABSTRACT: Uterine environment differs based on sex of developing fetuses. In prolific species, such as the sheep, co-twinning with a male fetus may subtly affect sexual differentiation of the female fetus and possibly life-time flock productivity. Lambing records from the University of Wyoming purebred sheep flocks were analyzed to determine if sex of a co-twin affects number of offspring, flock longevity, and age at first lambing. Breed differences were also evaluated. Nine years of lambing records (ewes born from 1995 – 2003) for Columbia, Hampshire, Rambouillet and Suffolk ewes (n = 547) were analyzed. Total number of lambs born to each ewe and number of years each ewe remained in the flock was evaluated. As expected number of lambs born ($P < 0.001$) and years in the flock ($P = 0.05$) was affected by breed, but there was no breed by co-twin sex interaction ($P > 0.4$). Suffolk ewes were the most productive with the most number of lambs and longest flock longevity. Flock longevity of Suffolk ewes did not differ ($P = 0.7$) from Rambouillet ewes. Columbia ewes had the fewest number of lambs, and shortest flock longevity, but flock longevity did not differ ($P = 0.9$) from Hampshire ewes. Rambouillet ewes produced the fewest number of offspring but remained in the flock longest. Number of lambs born, but not ($P = 0.22$) years ewes remained in the flock, tended ($P = 0.08$) to be affected by twinning. Ewes born as a single (n = 138) had fewer lambs during their productive lifetime than ewes born co-twin with ewes ($P = 0.05$; n = 193), but did not differ ($P = 0.8$) from ewes co-twined with rams (n = 216). To determine effect of co-twinning with a male, ewes born as singletons were removed from the data set. Presence of a male co-twin tended (P = 0.08) to decrease the number of lambs born but did not affect ($P= 0.13$) number of years a ewe remained in the flock. Sex of the co-twin did not affect ($P = 0.7$) age at first lambing. Ewe productivity tended to be affected by sex of the co-twin. This data suggests that flock productivity would benefit from selection of replacement ewes which are twinned with females.

Key Words: Co-twin sex, Productivity, Ewe.

Introduction

The presence of a male fetus in human twins changes the uterine growth environment for the co-twin female causing the female's birth weight to be greater than if she was co-twin to another female (Blumrosen et al., 2002). Three scales of typical male and female emotional behavior showed that females with a male co-twin behaved more like males than females co-twinned to another female (Loehlin et al., 1999). Gilts (Lamberson et al., 1988) and female rodents (vomSaal, 1981) born in litters with a large proportion of males also reach puberty later and have a shortened reproductive life.

Freemartinism is rare in livestock species other than cattle. In prolific species such as sheep, co-twinning with a male fetus results in a freemartin female only about 1-6.8% of the time and is more prevalent when litter numbers are four or more (Brace et al., 2008; Padula, 2005). Therefore, freemartinism in sheep is regarded as a rare, unimportant abnormality. Fitzgerald et al. (1993) reported that rams born co-twin to another male exhibited higher serving capacity scores than rams born co-twin to a female. Conversely, Price et al. (2000) reported that sexual performance and rates of mounting and ejaculation are no different between males co-twinned to males, males co-twinned to females or singles. Since females co-twinned to males has an effect on both birth weight and emotional characteristics in humans, age of puberty and reproductive life in swine, and serving capacity in rams, we hypothesized that ewes co-twinned to rams may reach puberty later and have a decreased reproductive life.

The objective of this study was to evaluate the effect of co-twin sex and breed on ewe productivity.

Materials and Methods

Animals. Lambing records from purebred Columbia, Hampshire, Rambouillet and Suffolk ewes (n = 547) from the University of Wyoming sheep flock were used for this study. Ewes were housed and lambed at the University of Wyoming livestock facilities.

Design and Data Analysis. The experiment utilized nine years of lambing records. Records included ewe identification, date of lambing, lamb number(s) and sex of lamb(s). Data was collected from replacement ewe lambs born from 1995-2003. Each ewe lamb was recorded as being a single (n = 138) or co-twin to another female (n = 193) or male (n = 216). Subsequently, lambing records for each ewe lamb were evaluated including total lambs birthed, number of years each ewe remained in the flock, and age at first lambing. Differences in ewe productivity due to birth-type, sex of co-sibling and breed were determined.

Statistics. All data were analyzed by GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The model included effects of breed and birthing type. In a second analysis, singles were removed from the data set to analyze effects of sex of co-twin.

Results

There was no breed by co-twin interaction (P > 0.4), but breed affected both number of lambs born (Table 1) and years ewes remained in the flock (Table 2). Suffolk ewes were the most productive with the most number of lambs born and longest flock longevity. Flock longevity of Suffolk ewes did not differ (P = 0.7) from Rambouillet ewes (Table 2). Columbia ewes had the fewest number of lambs and shortest flock longevity, which did not differ (P = 0.9) from Hampshire ewes (Table 2). Number of lambs born (Figure 1), but not years ewes remained in flock (Figure 2) tended to be affected by sex of a ewe's sibling. Ewes born as singles produced fewer lambs during their productive lifetime than ewes born co-twin with ewes (P = 0.05), but did not differ (P = 0.8) from ewes co-twinned with rams. To determine effect of co-twinning with a male, ewes born as singletons were removed from the data set. Presence of a male co-twin tended (P = 0.08) to decrease (4.84±0.21 vs. 5.39±0.22 for ewes co-twinned with male or female, respectively) the total number of lambs born but not (P = 0.13) number of years a ewe remained in the flock. Sex of the co-twin did not affect (P = 0.7) age at first lambing.

Discussion

Sex of a ewe's co-twin influenced her subsequent reproductive lifetime prolificacy by approximately 10%.

In cattle, freemartins occur in 92% of heterosexual twin pregnancies. However, freemartinism in sheep is rare, occurring in only about 1-6.8% twin pregnancies (Brace et al., 2008), even though the two species share a similar form of placentation.

One well-studied mechanism in livestock is the fusion of placental blood vessels between twins resulting in a common circulation (Brace et al., 2008). This potentially masculinized phenotype is primarily an effect of the circulating anti-Müllerian hormone and testosterone produced by the testes of the developing male (Brace et al., 2008). Effects on ewe productivity could be due to presence of male androgens in the uterus during pregnancy. Although ewes with male co-twins would not likely have testosterone concentrations sufficient to induce freemartinism, it is likely that they are exposed to higher levels of testosterone than ewes with female co-twins.

Breed differences were as expected. Lambs of British breeding, such as Suffolk and Hampshire, tend to reach puberty earlier than lambs with Spanish ancestry, such as Rambouillet and Columbia (Sheep Production Handbook, 1996). This would also explain why Suffolk and Hampshire ewes tended to breed in their first year of life while Rambouillet and Columbia did not. However, breeds such as Rambouillet have longer breeding seasons (Sheep Production Handbook, 1996) which does not explain why this breed had fewer total lambs than Suffolk and Hampshire ewes. Although white-face ewes are noted for their hardiness, differences in longevity did not differ from the black-face breeds in this farm flock management system.

Implications

Even though the propensity for twinning is a lowly heritable trait, it is economically important and easily selected for. Therefore, twin born lambs are often selected as replacement ewes. The current project, however, indicates that sex of a co-twin influences lifetime productivity of ewes.

Ewes born co-twin to a male lamb may produce approximately 10% less lambs during their productive lifetime than ewes born co-twin to a female lamb. More intense evaluation of further records is needed to determine if birth-type influences other aspects of production.

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Table 1. Breed effect on total lambs produced by each ewe.

	Mean	Std. Error
Columbia	4.25[a]	± 0.28
Hampshire	5.01[b]	± 0.25
Rambouillet	4.78[a,b]	± 0.25
Suffolk	5.92[c]	± 0.28

Columns with differing superscripts differ ($p < 0.05$)

Table 2. Breed effect on ewe longevity in the flock.

	Mean (yr.)	Std. Error
Columbia	3.69[a]	± 0.16
Hampshire	3.69[a]	± 0.14
Rambouillet	4.08[a,b]	± 0.14
Suffolk	4.15[b]	± 0.16

Columns with differing superscripts differ ($p < 0.05$)

Figure 1. Total number of lambs delivered by ewes born single, co-twin to a female or co-twin to a male fetus. Number of lambs tended (P = 0.08) to be effected by twinning.

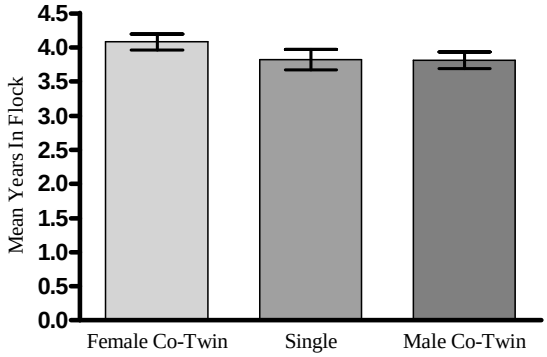

Figure 2. Flock longevity (yrs.) of ewes born single, co-twin to a female or co-twin to a male. Flock longevity was not (P = 0.22) different between groups.

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EFFECTS OF 8-EPI-PROSTAGLANDIN F2α AND PROSTAGLANDIN F2α ON PROGESTERONE CONCENTRATION AND CORPUS LUTEUM SIZE IN EWES

D. T. Yates, T. T. Ross, L. J. Yates, and D. M. Hallford
New Mexico State University, Las Cruces, NM

ABSTRACT: Prostaglandin F2α (PGF2α) is the major luteolysin in domestic livestock. The objective of the current study was to assess the luteolytic ability of a structural isomer of PGF2α, 8-epi-PGF2α. Ten mature Rambouillet ewes were synchronized with progesterone-impregnated vaginal inserts (CIDR) removed after 15 d. Blood serum was collected daily at 1300 from day of CIDR removal (d 0) to 12 d after removal for progesterone (P4) analysis (RIA). On d 8, ewes were injected with 0.1 mL ethanol, 200 µg PGF2α, or 200 µg 8-epi-PGF2α into the interstitial tissue of each ovary (via laparoscopy). On d 13, ewes were ovarectomized and corpora lutea were collected and weighed. Treatment by day interaction was found ($P < 0.05$) for P4, thus time responses were examined within treatment. In ewes injected with PGF2α, P4 decreased ($P < 0.05$) 24 h after administration, but began to rise again afterward. No effect ($P > 0.05$) on P4 was observed in ewes given ethanol or 8-epi-PGF2α. Luteal weights did not differ ($P > 0.05$) among treatments, indicating no structural luteolysis occurred as a result of treatment. These data indicate that a single intra-ovarian injection of 8-epi-PGF2α did not induce luteolysis in ewes. Additionally, a single dose of PGF2α only reduced P4 for 24 h before serum concentrations began to recover. Under the protocol of this experiment, multiple injections of the luteolysin may be necessary for complete luteolysis.

Keywords: 8-epi-prostaglandin F2α, luteolysis, prostaglandin F2α

INTRODUCTION

Early stages of gestation are influenced by the CL, which releases progesterone (P4) and allows a suitable environment for early embryonic development (Senger, 1999). Maintenance of the CL during early pregnancy in domestic ruminants occurs as a result of restriction of release of the luteolytic hormone, PGF2α, from the uterus onto the ovary; a process not restricted in non-gravid animals (Knobil and Neill, 1994). Release of PGF2α is biologically regulated. However, during periods of moderate to extreme environmental stress, secretion of 8-epi-PGF2α (a structural isomer of PGF2α) increases. This isomer is produced from arachodonic acid by an alternative and seemingly unregulated pathway involving free radicals as pseudo-enzymes (Takahashi et al., 1992; Klein et al., 1997; Yin et al., 2007). This novel isoprostane has been shown to share some functional properties of PGF2α, such as vasoconstriction (Takahashi et al., 1992), but its ability to act as a luteolysin has yet to be demonstrated. The objective of this study was to evaluate luteolytic effects of 8-epi-PGF2α when injected into the interstitial tissue of the ovary in luteal-phase ewes.

MATERIALS AND METHODS

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee (2008-027). Ten mature, multiparous Rambouillet ewes (70.0 ± 2.6 kg) were used to evaluate *in vivo* luteolytic ability of 8-epi-PGF2α in non-gravid sheep. Before use, animals were weighed, physically examined for health, and necks were shorn to ease blood collection. Animals had access to shelter and were fed chopped alfalfa hay (approximately 2 kg/ewe) once daily at 0600 and *ad libitum* water, unless otherwise stated. On d -14, all ewes received a progesterone-impregnated vaginal insert (EAZI-BREED CIDR, 0.3 g P4; Pharmacia and Upjohn, Co., Hamilton, New Zealand) to synchronize estrus. The CIDRs were removed on d 0 and a mature vasectomized ram equipped with a marking harness was introduced into the pen to detect estrus. Nine of the 10 ewes responded with standing heat on d 0 and the remaining ewe responded on d 1. At this time, the vasectomized ram was removed from the pen. Blood samples were collected once daily at 1300 from d 0 to 12 via jugular venipuncture (Corvac serum separator tubes). Samples were kept at room temperature for 30 to 60 min and then centrifuged (1,500 x g at 4° C for 15 min). After centrifugation, samples were stored at -80° C until ready for assay. Ewes were randomly assigned to 1 of 3 intra-ovarian treatments: 8-epi-PGF2α (200 µg in 0.1 mL absolute ethanol per ovary; BioMol International, LP, Plymouth Meeting, PA; Cat # PG-049), PGF2α (200 µg in 0.1 mL ethanol per ovary; Lutalyse, Pfizer, Inc., New York, NY), or ethanol placebo (0.1 mL ethanol per ovary). On d 8, treatment was injected into the interstitial tissue of both ovaries in each ewe via laparoscopy. Ewes were held off feed and water for 18 h before laparoscopic procedures. Just before beginning the procedure, each ewe was given general anesthesia (Ketamine, 1 mL, i.v.), the surgery site was washed and prepped, and the ewe transferred to a wheeled laparoscopy gurney. Lidocaine (5 mL, s.c.) was administered in the abdomen, approximately 3 cm to either side of the mid-line and 2 cm anterior to the mammary gland. A 1-cm incision was made through the skin and a trocar was used to insert a 6-mm laparoscopic cannula through the abdominal wall and into the abdominal cavity at the site of the right-side incision. Carbon dioxide gas was slowly pumped through

the cannula to moderately inflate the abdominal cavity. After inflation, a second trocar was used to insert a 10-mm cannula at the site of the left incision, and the laparoscopic scope (GYRUS XLS-300, ACMI Corp., Southborough, MA) was inserted into the second cannula to locate the ovaries. A needle (18 gauge, 60 cm) was inserted through the first cannula and into the center of the interstitial tissue of the ovary. An assistant then pushed the prescribed treatment through the needle and into the ovary using a 1 mL syringe. Injected fluid was followed by a predetermined amount of air to displace any fluid that may have remained in the needle. The procedure was immediately repeated for the second ovary, regardless of appearance or absence of a CL on either or both ovaries. Animals were then returned to their original pens and closely monitored for 24 h. On d 14, ovarectomies were performed on all ewes. Feed and water was restricted for 24 h before ovarectomies. Ewes were anesthetized with Ketamine (1 mL, i.v.), surgical sites were washed and prepped, and ewes were transferred to a wheeled surgical gurney, restrained, and transported to the surgical room, where ovaries were removed by trained technicians using an IACUC-approved protocol under sustained general anesthesia (Ketamine, 1 ml, i.v., approximately once every 15 min). After removal of the ovaries, animals were given penicillin (5 mL, s.c.), flunixine meglumine (Flunix, 2.5 mL, i.m.), and topical antimicrobial and insecticide treatments, and were offered one-fourth alfalfa rations and *ad libitum* water for 3 d. Serum samples were analyzed for P4 concentration via RIA (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA; intra-assay CV = 8.7 %; single assay; Schneider and Hallford, 1996) to indicate functional luteolysis. Corpora lutea were removed from each ovary and weighed as an indication of structural luteolysis.

Ewe was considered the experimental unit. Experimental design was a completely random design for CL data and split plot for P4 concentrations (whole plot = treatment; sub plot = day). Treatment structure was one-way with 3 classes: 8-epi-PGF2α, PGF2α, and ethanol control. All data were analyzed by SAS (SAS Inst. Inc., Cary, NC). Differences among treatment groups for CL data were examined using the GLM procedure. Progesterone data were analyzed for differences among treatments, differences among days, and treatment by day interaction using the GLM procedure of SAS. Pair-wise comparisons were made among treatments within each day and among days within each treatment using Bonferroni's t-test.

RESULTS

An interaction ($P < 0.01$) between treatment and day was observed for serum P4 concentration. When examined by day, P4 did not differ ($P > 0.05$) due to treatment on any day. As expected, P4 differed ($P < 0.01$) among days within all 3 treatment groups (Figure 1). In ewes given PGF2α on d 8, P4 decreased ($P < 0.05$) from 4.0 to 1.4 ± 0.4 ng/mL from d 8 (day of treatment) to d 9, but then recovered by increasing ($P < 0.05$) to 4.2 ± 0.4 ng/mL on d 12. No decrease ($P > 0.05$) was observed after injection in ewes given ethanol or 8-epi-PGF2α, and P4 concentrations were greater ($P < 0.05$) on d 12 and 11, respectively, than on day of treatment for these treatment groups. Serum P4 concentration peaked on d 11 in ewes receiving ethanol and on d 12 in those given PGF2α or 8-epi-PGF2α. Average CL weight per ewe and total CL weight per ewe did not differ ($P > 0.05$) among treatments (Table 1). Blocking by number of CL was not possible, as all ewes randomly assigned ethanol had 2 CL. Due to minimal amount of replication, data from this study should be interpreted with caution.

Table 1. Corpus luteum weights (g) in Rambouillet ewes administered ethanol, 8-epi-PGF2α, or PGF2α via injection into interstitial tissue of each ovary on d 8 after removal of P4 insert.

Item	Ethanol	8-epi-PGF2α	PGF2α	SE
Average CL weight[1, 2]	0.62	0.59	0.54	0.02
Total CL weight[1, 3]	1.24	1.02	1.10	0.14

[1]Row values do not differ ($P > 0.05$).
[2]Mean CL weights per ewe were average within treatment.
[3]Total CL weight per ewe was averaged within treatment.

DISCUSSION

Data collected from this experiment do not indicate that 8-epi-PGF2α possesses the same luteolytic ability of PGF2α. Although very little data was previously available on luteolytic ability of this isoprostane, Weems et al. (1998a) described a similar relationship between another prostaglandin, PGE2, and its structural isomer, 8-epi-PGE2, by demonstrating that 8-epi-PGE2 did not function like PGE2 in the CL of sheep. However, inability of 8-epi-PGF2α to influence P4 in the current study was a contrast to other findings by Weems et al. (1998b), which indicated an ability of the isoprostane, when exogenously administered, to alter the PGE to PGF2α ratio within luteal tissue. Because this ratio appears to at least partially govern P4 production (Weems et al., 1997), output of P4 was affected. Other studies have reported shared functions between PGF2α and 8-epi-PGF2α. Aghajanian et al. (1997) described a powerful vasoconstrictive ability of 8-epi-PGF2α, a widely known function of PGF2α. However, this shared function may not be facilitated through a common receptor. In fact, recent data indicate the vasoconstrictive ability of 8-epi-PGF2α actually results from its ability to bind to the receptor of thromboxane A2 (Comporti et al., 2008), although this type of binding is disputed by earlier work, which described the isoprostane as having no effect on the thromboxane receptor (Weber and Markillie, 2003). Additionally, Unmack et al. (2001) suggested that 8-epi-PGF2α acted on prostanoid E receptors rather than thromboxane A2 receptors, at least in the porcine small intestine. This assertion corroborated earlier work indicating compatibility between 8-epi-PGF2α and the prostanoid E receptor (Elmhurst et al., 1997). Certainly,

the ability of 8-epi-PGF2α to bind and activate receptors of vasoconstrictors other than PGF2α could explain its ability to mimic this property of PGF2α while failing to induce other known functions of the prostaglandin, including luteolysis.

Although the ability of 8-epi-PGF2α to bind and activate the receptor of PGF2α has been neither clearly demonstrated nor refuted, it is possible that reduced receptor affinity or binding ability could lead to a diminished response to 8-epi-PGF2α compared to similar amounts of PGF2α. In any case, 8-epi-PGF2α administered at 200 µg per ovary failed to induce either structural or functional luteolysis. Interestingly, structural integrity of the CL was not affected by injection of a known luteolysin, PGF2α, despite its apparent ability to temporarily diminish P4 output when administered at 200 µg per ovary. Reduction of P4 concentration 24 h after injection coupled with the recovery of P4 concentration within a few days after injection seem to indicate that only a portion of luteal cells were responsive to the introduction of luteolysin into the interstitial tissue of the ovary. Support for this possibility is suggested by the findings of Shirasuna et al. (2007), which demonstrated that mature, fully luteinized luteal cells of bovine CL were more responsive to PGF2α than those still in the process of luteinization. Functional and structural luteolysis of ovine CL in response to PGF2α or any other proposed luteolyte may be more fully demonstrated or refuted if the intra-ovarian model used in the current study were adjusted to include serial administration and increased dosage.

IMPLICATIONS

Data did not indicate that 8-epi-PGF2α is luteolytic at the dosage administered. Increased dosage, repeated administration, or a combination of the two may have a different effect on structure or function of the CL. Intra-ovarian injection of PGF2α only temporarily diminished P4 concentration, but concentrations recovered within 4 d of administration, indicating incomplete sensitivity to PGF2α by luteal tissue on d 8 after CIDR removal. Prostaglandin F2α administered at d 8 did not affect CL structure.

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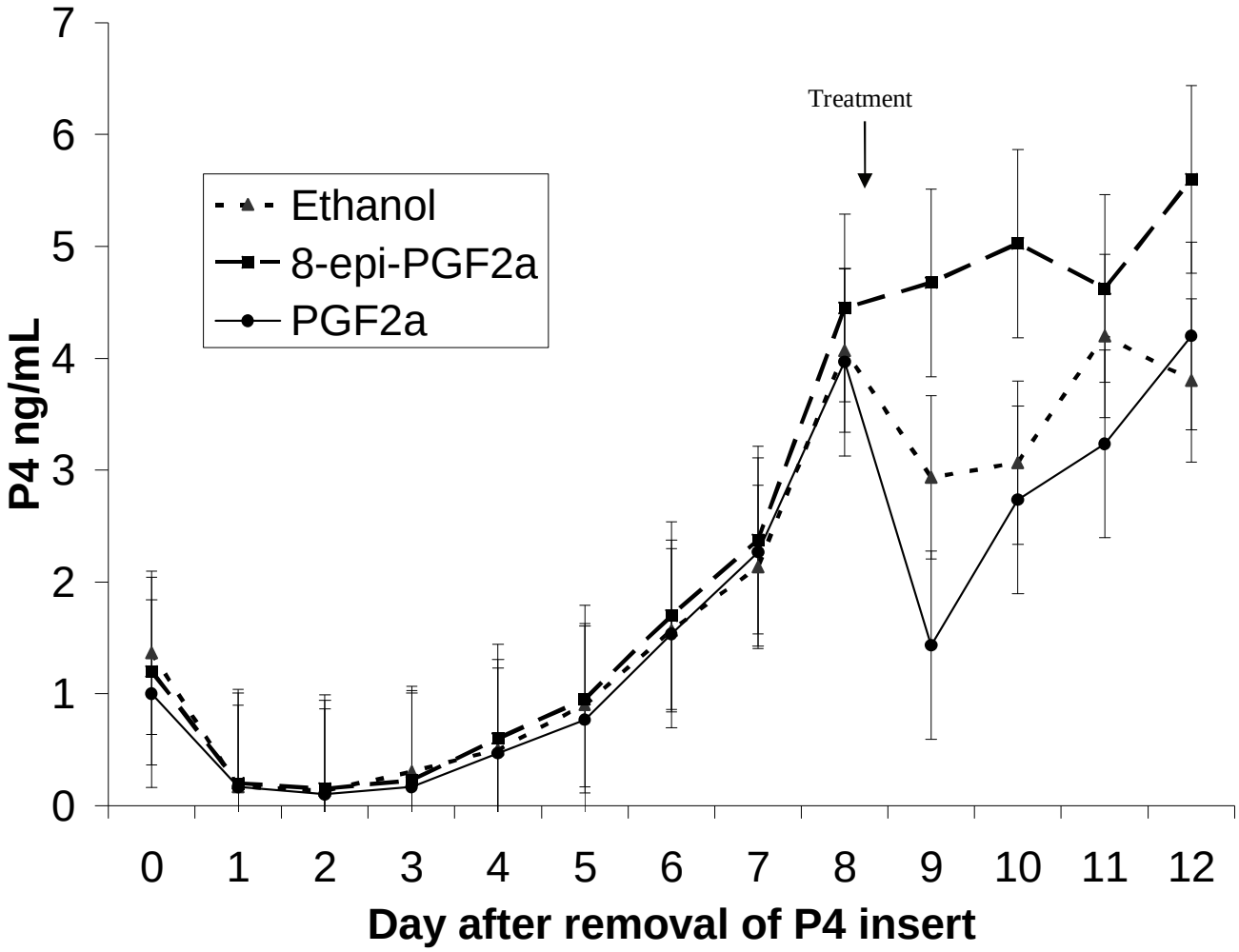

Figure 1. Progesterone (P4) concentrations (ng/mL) of Rambouillet ewes administered ethanol, 8-epi-PGF2α, or PGF2α via injection into interstitial tissue of each ovary on d 8 after removal of P4 insert (treatment by day, $P < 0.01$).

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EFFECTS OF HUMAN CHORIONIC GONADOTROPIN ON SERUM PROGESTERONE CONCENTRATION AND COMPLETE BLOOD COUNTS DURING EARLY GESTATION IN EWES

D. T. Yates, T. T. Ross, L. J. Yates, A. R. Otis, C. A. Warner, and D.M. Hallford
New Mexico State University, Las Cruces, NM

ABSTRACT: Luteal maturation and progesterone (P4) production has been implicated in embryonic survival during early stages of pregnancy, as it promotes uterine quiescence and may temper immune response against the embryo. The current study examined effects of hCG on serum P4 concentration and complete blood counts (CBC) when administered at various stages over the first 2 wk after mating. In Exp. 1, 46 ewes (60.6 ± 1.8 kg) were synchronized (P4 insert) and hand-mated at detected estrus (vasectomized ram, d 0). Ewes were given (i.m.) 0 (saline, CON) or 100 IU hCG on d 4 (hCG-4) or 8 (hCG-8). Progesterone was measured on d 3 to 13, 15, 17, 19, 21, and 25. In Exp. 2, 56 ewes (64.0 ± 1.2 kg) were synchronized (P4 insert) and naturally mated in groups, then administered (i.m.) hCG (hCG) or saline (CON) on d 4, 7, 10, and 13 after estrus. Progesterone was measured on d 4 to 15 and CBC on d 7 and 11. In Exp. 1, P4 was greater ($P < 0.05$) in hCG-8 than in CON for d 9, 10, and 11 only. No difference ($P > 0.05$) in P4 was found between hCG-4 and CON or between hCG-4 and hCG-8 on any day. In Exp. 2, P4 was greater ($P < 0.05$) in hCG than in CON on d 9, 11, 12, 13, 14, and 15. On d 7, total white blood cells (WBC) and total lymphocytes were greater ($P < 0.05$) in hCG than in CON. Likewise, mean corpuscular volume ($P = 0.06$) and number of neutrophils ($P = 0.07$) tended to be greater while eosinophil fraction of WBC tended to be less ($P = 0.06$) in hCG ewes compared with CON values. On d 11, red blood cells, hemoglobin, and hematocrit were reduced ($P < 0.05$) in hCG compared to CON. Data indicate that administration of hCG during early gestation increases serum P4 and influences components of CBC, and that degree of influence depends largely on timing of hCG administration.

Keywords: Complete blood counts, human chorionic gonadotropin, progesterone

INTRODUCTION

Progesterone (P4) insufficiency during embryonic implantation (McLaren, 1973), maternal immune response toward the conceptus (Howell et al., 1994), or a combination of these and other factors may contribute to early embryonic mortality. Providing exogenous luteotropic hormones has been shown to increase P4 concentration during early pregnancy and, ultimately, reduce embryonic death (Willard et al., 2003). Lessening maternal immune attack on newly formed concepti could further protect embryogenesis during pre-implantation (Szekeres-Bartho, 1990). Human chorionic gonadotropin (hCG) increases P4 in domestic livestock similarly to LH (Szmidt et al., 2008) and may reduce maternal immune response against developing embryos (Khil et al., 2007). The objective of this study was to determine the effects of hCG administered to sheep at various times during the first weeks of pregnancy on serum P4 concentration and immune components of complete blood counts (CBC).

MATERIALS AND METHODS

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee (2007-022; 2008-024). Before use, ewes were weighed and examined for health, and necks were shorn to ease blood collection. For the duration of the experimental period, animals had access to water and shelter and were fed chopped alfalfa hay (approximately 2 kg/head) once daily at 0600.

Experiment 1.

Fifty-seven mature Suffolk ewes (60.6 kg ± 1.8) were used to explore the effect of a single, timed injection of hCG on P4 concentration during early pregnancy. On d -15, all ewes received a P4-impregnated vaginal insert (EAZI-BREED CIDR, 0.3 g P4; Pharmacia and Upjohn, Co., Hamilton, New Zealand) to synchronize estrus. Inserts were removed on d -1 and 2 mature vasectomized rams fitted with marking harnesses were released into the flock to detect estrus. Forty-four ewes responded with standing heat on d 0, a single ewe responded at d 1, and 12 ewes were not marked by the vasectomized rams and were removed from the experiment. Ewes were checked for mounting marks twice daily (0800 and 1800), and at each check, marked ewes were pulled from the flock and paired individually with 1 of 3 randomly assigned Suffolk rams in a 3 by 3 m pen. Rams were allowed to fully copulate with each ewe twice. After the second mount, ewes were returned to the flock, and hand-mating was repeated 12 h later. Rams were allowed a minimum of 5 min between ewes for recovery. Ewes were randomly assigned to 1 of 3 treatment groups: those receiving hCG (ProSpec-Tanny TechnoGene, Ltd, Rehovot, Israel, CAS: HOR-250) on d 4 (hCG-4; 100 IU in 1 mL physiological saline, i.m.; n = 15), those receiving a similar injection of hCG on d 8 (hCG-8; n = 16), and those receiving saline placebo (CON; 1 mL physiological saline, i.m.) on either d 4 or 8 (n = 15). Serum samples were collected once daily at 1800 on d 3 to 13, 15, 17, 19, 21, 23, and 25 via jugular venipuncture (Corvac serum separator tubes) and assayed for P4 concentration.

Experiment 2.

Forty-two multiparous Suffolk ewes and 18 nulliparous long-yearlings (64.0 kg ± 1.2) were used to determine effects of repeated injections of hCG during the first 2 wk of pregnancy on P4 concentration and CBC. On d -15, all ewes received a P4-impregnated vaginal insert (EAZI-BREED CIDR, 0.3 g P4; Pharmacia and Upjohn, Co., Hamilton, New Zealand) to synchronize estrus. Inserts were removed on d -1 and ewes were divided randomly into 4 breeding groups. Beginning at d 0, each group was held in an isolated pen for 48 h with an assigned ram fitted with a marking harness. After the 48-h mating period, rams were removed. Ewes were assigned randomly to 1 of 2 treatment groups: those receiving repeated, timed injections of hCG (hCG; 100 IU in 1 mL physiological saline, i.m.; ProSpec-Tanny TechnoGene, Ltd, Rehovot, Israel, CAS: HOR-250) or saline placebo (CON; 1 mL physiological saline, i.m.). Injections were administered at 0700 on d 4, 7, 10, and 13. Serum samples were collected once daily at 0700 on d 3 to 15 via jugular venipuncture (Corvac serum separator tubes) and assayed for P4 concentration. Whole blood samples were also collected at 0700 on d 7 and 11 (EDTA-containing whole-blood vacuum tubes). Immediately after sampling, whole-blood samples were packaged on ice and shipped overnight to the Veterinary Diagnostics Services, Albuquerque, NM, for CBC analysis: white blood cell concentration (WBC), red blood cell concentration (RBC), hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet concentration, and absolute number and fraction of total WBC of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils (BAS). On d 15, all ewes received a cautionary dose of liquamyacin (5 mL, s.c.; LA-200, Pfizer, Inc., New York, NY), and were returned to the flock for the remainder of gestation.

Progesterone Assay. Blood samples were kept at room temperature for 30 to 60 min and then centrifuged (1,500 x g at 4° C for 15 min). After centrifugation, serum samples were stored at -80° C until ready for assay. Radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA; Schneider and Hallford, 1996) was used to analyze P4 concentration in all serum samples (Exp. 1 mean intra-assay CV = 5.3 over 4 assays; inter-assay CV = 13.8%; Exp. 2 mean intra-assay CV = 6.5 % over 5 assays; inter-assay CV = 5.9 %).

Statistical Analysis. Individual ewes were experimental units. Experimental design was a split-plot (whole-plot = treatment, sub-plot = day). Treatment design was one-way with 3 classes (hCG-4, hCG-8, and CON) for Exp. 1 and 2 classes (hCG and CON) in Exp. 2. All data were analyzed by SAS (SAS Inst. Inc., Cary, NC). Progesterone and CBC were analyzed for differences among treatments, differences among days, and treatment by day interaction using the GLM procedure. The pdiff function was used for mean separation in Exp. 1.

RESULTS

Data from Exp. 1 (Figure 1) show increased ($P < 0.05$) serum P4 concentration on d 9, 10, and 11 in hCG-8 ewes compared to CON ewes. Progesterone concentration did not differ ($P > 0.05$) between hCG-4 and CON or between hCG-4 and hCG-8 ewes on any day. Increased P4 concentration in hCG-8 ewes lasted approximately 72 h before P4 concentrations returned to levels similar to CON ewes. In Exp. 2, P4 concentration (Figure 2) was greater ($P < 0.05$) in hCG ewes than in CON ewes on d 9, 11, 12, 13, 14, and 15, but did not differ ($P > 0.05$) between hCG and CON ewes on d 4, 5, 6, 7, 8, or 10.

On d 7, WBC and LYM (Table 1) were in greater concentration ($P < 0.05$) in the blood in hCG ewes than in CON. Additionally, MCV ($P = 0.06$) and NEU ($P = 0.07$) tended to be greater in hCG ewes and EOS fraction of WBC tended to be reduced ($P = 0.06$) in hCG ewes compared to CON ewes. On d 11 (Table 2) RBC, Hgb, and Hct were reduced ($P < 0.05$) in hCG ewes compared to CON ewes. Additionally, WBC, LYM, MCV, EOS fraction of total WBC, and NEU which differed between treatments on d 7 ($P < 0.05$) did not differ at d 11 ($P > 0.05$).

DISCUSSION

Data from this study support the hypothesis that hCG administration to ewes during early pregnancy can enhance production of P4, as evidenced by increased serum P4 in ewes injected with hCG. However, this response appears largely dependant on timing of treatment. Earlier work indicated that hCG given too early after ovulation may not affect luteal production of P4 (Breuel et al, 1989). Evidence from the current study supports these previous findings, as injection of hCG on d 4 did not increase serum P4 in either experiment. Seemingly, developing luteal cells of the CL must reach a certain degree of maturity before they are responsive to the stimulatory effects of hCG. Data from Exp. 2 indicate that d 8 is the approximate time at which luteal cells have reached a level of maturation that allows a steroidogenic response to hCG, as serum P4 concentration was increased in days subsequent to this injection, but not before, despite previous injections of hCG. Data from Exp. 1 support the concept of a d 8 threshold of luteal cell maturation, as d 8 injections of hCG in this experiment increased serum P4 as well. Although hCG half-life in circulation has been reported as approximately 22 h (Schmitt et al., 1996), data from Exp. 1 indicate that a single dose of hCG given at d 8 elicited increased serum P4 for about 72 h, and in Exp. 2, hCG injections administered 72 h apart held the increase from d 9 through the end of the collection period. The latter observation suggests no desensitization of the luteal cells to hCG over this time period, an inference that supports similar findings in cattle (Helmer and Britt, 1987).

Data from d 7 CBC in Exp. 2 disagree with previous work that reported an inhibitory effect of hCG on LYM activity (French and Northey, 1983), as LYM numbers were actually greater in hCG-treated ewes in the

current study. Interestingly, however, LYM numbers in ewes receiving hCG returned to control levels by d 11, indicating changing dynamics of the influence of hCG on immune response. Additionally, hCG appeared to have no effect on MON population, either in total numbers or as a percentage of total WBC and at either d 7 or d 11, as previously reported in mice (Khil et al. 2007). In fact, the overall increase in total WBC and LYM numbers at d 7 and lack of difference in any component of WBC at d 11 is contradictory to the idea of immune depression by hCG, although EOS fraction of total WBC was decreased at d 7. These data may lend support, however, to the suggestion of French and Northey (1983) that changes in immune response dynamics toward the embryo are confined to the location of the uterus.

Decreased RBC, Hgb, and Hct recorded at d 11 in hCG ewes is in contrast to findings in human males treated with hCG, which revealed increased levels of these 3 blood components (Tsujimura et al., 2005). Plotka et al. (1988) showed a correlation between P4 levels and RBC, Hgb, and Hct levels in wild horses, but attributed this phenomenon to overall health and condition of the animals. Clearly, the link between hCG administration and RBC, Hgb, and Hct levels is poorly understood, but the effect of hCG on these blood components was noticeably absent until after increased serum P4 concentration was observed.

IMPLICATIONS

Human chorionic gonadotropin was shown to enhance natural progesterone production in ewes, but only after a threshold level of luteal cell maturity was reached at approximately d 8. Before this time, hCG treatment did not improve progesterone production. However, WBC, lymphocytes, MCV, and eosinophil percentage of WBC were increased and neutrophils were decreased by hCG before the appearance of increased P4, but all returned to control levels by d 11. Also, RBC, hemoglobin, and hematocrit levels were decreased after progesterone augmentation after being unchanged prior, but the mechanism and physiological significance of this change remains unclear.

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Table 1. Components of d 7 post-mating complete blood counts in Suffolk ewes injected with hCG or saline on d 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-val
White blood[2] cells	12.20	23.34	2.17	< 0.01
Red blood[3] cells	10.96	10.25	0.22	0.11
Hemoglobin[4]	11.96	11.58	0.25	0.47
Hematocrit[5]	33.00	32.11	0.72	0.55
MCV[6]	30.22	31.44	0.33	0.06
MCHC[4]	36.13	35.98	0.12	0.52
Neutrophils[5]	33.33	37.33	2.95	0.51
Lymphocytes[5]	55.44	54.89	2.91	0.93
Monocytes[5]	5.78	4.78	0.85	0.57
Eosinophils[5]	5.22	2.33	0.80	0.06
Basophils[5]	0.22	0.22	0.10	0.99
Platelets[2]	240.71	187.86	26.34	0.33
Absolute[2] Neutrophils	4.12	8.24	1.15	0.07
Absolute[2] Lymphocytes	6.69	13.63	1.25	< 0.01
Absolute[2] Monocytes	0.69	1.00	0.20	0.47
Absolute[2] Eosinophils	0.67	0.40	0.11	0.24
Absolute[2] Basophils	0.03	0.07	0.02	0.45

[1]Standard error (n = 15).
[2]Count * 10^3.
[3]Count * 10^6.
[4]g/dL.
[5]Percentage of total WBC.
[6]fL.

Table 2. Components of d 11 post-mating complete blood counts in Suffolk ewes injected with hCG or saline on d 4, 7, 10, and 13 post-mating.

Item	Control	hCG	SE[1]	*P*-val
White blood[2] cells	15.41	19.32	1.58	0.22
Red blood[3] cells	11.01	9.77	0.24	< 0.01
Hemoglobin[4]	11.99	10.80	0.26	0.01
Hematocrit[5]	34.22	31.22	0.79	0.05
MCV[6]	31.00	34.86	0.36	0.17
MCHC[4]	35.16	34.86	0.21	0.50
Neutrophils[5]	32.89	28.22	2.10	0.27
Lymphocytes[5]	59.78	66.33	2.25	0.15
Monocytes[5]	3.56	2.11	0.55	0.19
Eosinophils[5]	3.78	3.22	0.72	0.71
Basophils[5]	0.00	0.11	0.06	0.33
Platelets[2]	205.80	243.80	20.06	0.37
Absolute[2] Neutrophils	5.22	5.04	0.59	0.88
Absolute[2] Lymphocytes	9.10	13.23	1.24	0.09
Absolute[2] Monocytes	0.58	0.43	0.10	0.49
Absolute[2] Eosinophils	0.51	0.3	0.08	0.89
Absolute[2] Basophils	0.00	0.03	0.02	0.33

[1]Standard error (n = 21).
[2]Count * 10^3.
[3]Count * 10^6.
[4]g/dL.
[5]Percentage of total WBC.
[6]fL.

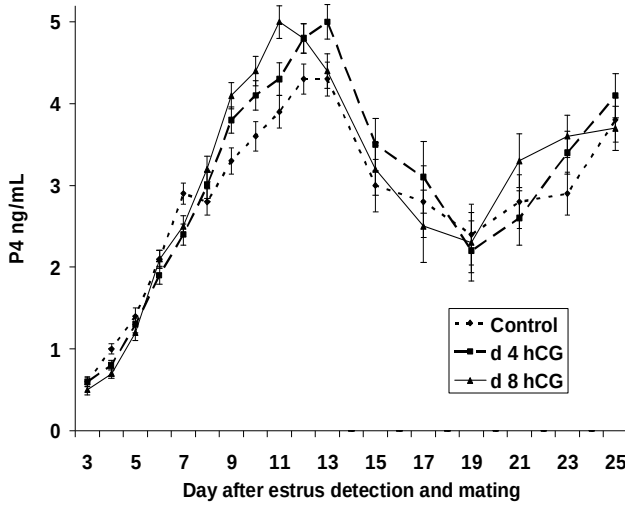

Figure 1. Serum progesterone concentrations (ng/mL) in Suffolk ewes administered hCG or saline on d 4 or 8 after estrus detection and mating (effect for treatment, $P > 0.05$, effect for day, $P < 0.05$, treatment by day, $P = 0.98$).

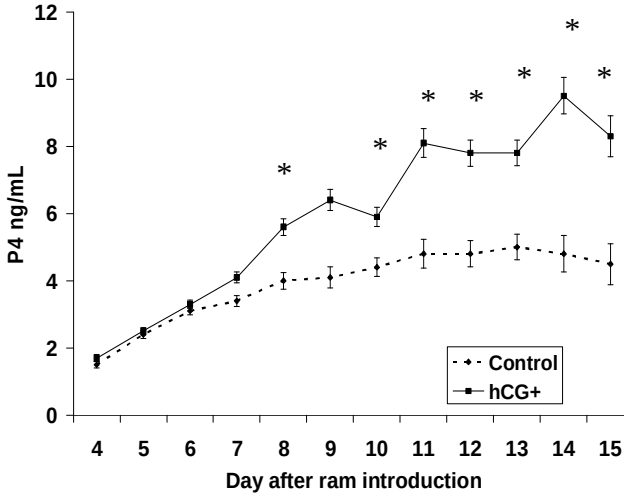

Figure 2. Serum progesterone concentration (ng/mL) in Suffolk ewes administered hCG or saline on d 4, 7, 10, and 13 after introduction of ram for mating (treatment by day, $P < 0.01$; * denotes treatment differences, $P < 0.05$).

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SERUM PROGESTERONE PROFILES AND CONCEPTION RATES IN RAMBOUILLET EWES TREATED WITH INTRAVAGINAL PROGESTERONE AT TWO STAGES OF THE ESTROUS CYCLE

A. N. Iroz, L. E. Camacho, A. D. Stapp, K. A. Jurado, M. B. Horvath, and D. M. Hallford
New Mexico State University, Las Cruces

ABSTRACT: Sixty mature Rambouillet ewes (64 ± 1.0 kg) were used to examine effects of estrus synchronization at 2 stages of the estrous cycle on serum progesterone (P4) profiles and conception rates. The study was conducted during a normal fall breeding season and ewes were maintained in an outdoor pen (12 x 18 m) with access to alfalfa hay (1.8 kg/ ewe daily) water, salt, and shade. On the day of estrus (d 0, determined by vasectomized rams), ewes were randomly assigned to 1 of 3 treatments. Twenty ewes served as untreated controls while remaining ewes received an intravaginal insert containing 0.3g of P4 (CIDR) on either d 0 (n = 20) or d 8 (n = 20) of the estrous cycle. The CIDR inserts were removed after 12 d. Blood samples were collected from all ewes from d 0 through d 15 and serum P4 was determined by RIA. Control ewes were placed with fertile Rambouillet rams on d 15 and CIDR-treated ewes were joined with rams on the day of CIDR removal. Pregnancy was determined by ultrasonography at 3.5 mo of gestation. Serum P4 was similar (P = 0.12) among treatments on d 0. On d 1, serum P_4 was 4.1 ± 0.1 ng/mL in ewes that received a CIDR on d 0 compared with 0.3 ng/mL in the other 2 groups (P < 0.001). Ewes in the d 0 group continued to have greater (P < 0.01) serum P4 than those in the other groups through d 8. On d 9, serum P4 in control and d 0 ewes was similar but was elevated (P < 0.01) in ewes that received a CIDR on d 8 (6.0 = 7.2 < 9.7 ± 0.5 ng/mL, respectively). This P4 relationship continued through d 12 after which the CIDR was removed from d 0 ewes and serum P4 values differed (P < 0.001) among all 3 treatments (0.4 < 6.3 < 8.8 ± 0.4 ng/mL for d 0, control, and d 8 ewes, respectively). Pregnancy rates were 70, 80, and 75% at the first estrus after CIDR removal in control, d 0, and d 8 ewes, respectively (P = 0.77). Insertion of a P4 CIDR on the day of estrus did not result in decreased conception compared with controls or ewes in which the CIDR was inserted during the luteal phase of the estrous cycle.

Key words: reproduction, sheep, synchronize

INTRODUCTION

Estrus synchronization and the ability to manipulate or initiate onset of estrus in ewes would economically benefit the sheep industry by consolidating labor needed for lambing, creating a more uniform lamb crop, and allowing producers to fill seasonal demands in the lamb market. Treatment of ewes with exogenous progesterone (**P4**) decreases days to estrus and allows synchronization of ewes by decreasing days to the first peak of prostaglandin F2α (Ottobre et al., 1980). Use of controlled internal drug releasing devices (**CIDR**) is a method of P4 delivery in cattle but is not yet available to US sheep producers. Progesterone delivery by CIDR has been shown to effectively increase serum P4 concentrations in the ewe (Ainsworth and Downey, 1986; Wheaton et al., 1993; Gifford et al., 2003), shorten days to estrus (Woody et al., 1967), and induce estrus in anestrous ewes (Knights et al., 2001). Following removal of the CIDR, P4 concentrations return to pre-CIDR values (Duffey et al., 2003). However, CIDR use may be detrimental to conception rate in ewes when inserted on the day of estrus (Duffey, 2003). This suggests a possible adverse effect on the CL when P4 is administered early in the estrous cycle. Loy et al. (1960) demonstrated that size of the CL was decreased by exogenous P4 early in the estrous cycle of cattle. Luteinizing hormone concentrations were also depressed by use of exogenous P4 early in the estrous cycle (Ottobre et al., 1980). The objective of the current study was to evaluate CIDR effects on conception rates in mature Rambouillet ewes treated with CIDR either on the day of estrus or 8 d after onset of estrus (luteal phase).

MATERIALS AND METHODS

All animal procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Sixty Rambouillet ewes (64 ± 1.0 kg), were maintained in an outdoor pen (12 x 18 m) during a normal fall breeding season with access to alfalfa hay (1.8 kg/ ewe daily), water, salt, and shade. Day of estrus was determined using 3 mature vasectomized Rambouillet rams equipped with marking paint. On the morning of first detected estrus (d 0) ewes were randomly assigned to 1 of 3 groups and blood was collected into sterile vacuum tubes (Corvac 7, Kendall Health Care, St. Louis, MO) via jugular venipuncture. Following blood collection, P4-containing intravaginal devices (CIDR 0.3g P4, Pharmacia and Upjohn Pty Limited, Rydalmere, NSW) were inserted on either d 0 (n = 20) or d 8 (n = 20) of the estrous cycle. Control ewes (n = 20) did not receive a CIDR. All CIDR remained in place for 12 d.

Blood was collected before morning feeding until d 15 of the estrous cycle. On d 12, blood was collected before the CIDR was removed. The CIDR-treated ewes were joined with fertile Rambouillet rams on the day of CIDR removal while control ewes were joined with rams on d 15. Ewes remained with rams for a 5-d breeding period. Blood samples were allowed to clot for 30 min at room temperature after which serum was separated by centrifugation at 1,500 x g for 15 min at 4° C and stored in plastic screw top vials at -20° C. Serum P4 concentrations were quantified by RIA using a solid phase kit (Siemens

Medical Solutions Diagnostics, Los Angeles, CA; Coat-A-Count) with modifications as reported by Schneider and Hallford (1996). Both within and between assay CV were less then 13%. Ewe pregnancy status was determined by ultrasonography at approximately 3.5 mo of gestation. Effects of treatment on serum P4 concentrations were evaluated using repeated measures analysis for a split plot design. Treatment effects were included in the main plot and day of sampling and treatment by day interactions were included in the subplot. Analyses were computed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Pregnancy rates were examined using the frequency procedure of SAS.

RESULTS AND DISCUSSION

Before the study began, ewe BW averaged 64 ± 1.0 kg. At the end of the experiment, control ewes weighed 67.1 kg compared to 68.7 and 64.2 (± 1.9) kg for those that received a CIDR on either d 0 or d 8 of an estrous cycle, respectively (P = 0.25). Animal weights were, therefore, not affected by the P4-containing inserts.

Serum P4 profiles of ewes in the 3 treatment groups are presented in Figure 1. As mentioned previously, day of estrus (d 0) was determined by raddled vasectomized rams. Data shown in Figure 1, therefore, are expressed by actual day of the estrous cycle and CIDR were inserted on either d 0 or d 8 in 2 of the treatment groups. A treatment by day interaction was detected (P < 0.001) necessitating examination of treatment effects within day. Serum P4 was approximately 0.5 ng/mL in the 3 groups on d 0. On d 1 ewes that received a CIDR on d 0 had 4.1 ± 0.1 ng/mL of P4 compared with about 0.4 ng/mL in the other 2 groups (P < 0.001). On d 7 of the estrous cycle, ewes in the d 0 CIDR group had a serum P4 concentration of 6.7 ± 0.4 ng/mL with 4.4 and 3.9 (± 0.4) ng/mL in control and d 8 ewes, respectively (P < 0.001). This same P4 level relationship was also present on d 8 before ewes in the d 8 group received a CIDR.

On d 9, control ewes and those in the d 0 group had similar P4 concentrations while those in the d 8 group had elevated P4 values (6.0 = 7.2 < 9.7 ± 0.5 ng/mL, respectively; P < 0.001). On d 12 of the estrous cycle before CIDR removal in the d 0 ewes, P4 values were again similar in control (6.8 ± 0.5 ng/mL) and d 0 (6.0 ± 0.5 ng/mL) ewes but those in the d 8 group had greater (P < 0.001) serum P4 (10.5 ± 0.5 ng/mL) than in the other treatments.

On d 13, serum P4 values were 7.2, 2.8, and 10.6 (± 0.5) ng/mL in control, d 0, and d 8 ewes, respectively(P < 0.001). Likewise, serum P4 differed (P < 0.001) among all 3 groups on d 15 (0.4 < 6.3 < 8.8 ± 0.4 ng/mL for d 0, control, and d 8 ewes, respectively). These data demonstrate that insertion of CIDR on d 0 effectively elevated serum P4 over controls for about 8 d. From d 9 through 12, serum P4 in control and d 0 ewes were similar demonstrating that CIDR insertion on d 0 did not elevate serum P4 above the level contributed by the mid-luteal CL. However, if the CIDR was inserted on d 8 when the CL was producing significant amounts of P4, serum P4 was actually greater than that observed in controls. Duffey et al. (2003) reported similar P4 profiles for controls and ewes receiving a CIDR on d 0 of an estrous cycle. Likewise, Gifford et al. (2003) showed a dramatic decline in serum P4 concentration within 12 h after CIDR removal from ovariectomized ewes.

As mentioned previously, pregnancy rates were determined by ultrasonography at approximately 3.5 mo of gestation. Pregnancy rates at the first estrus after CIDR removal were 70, 80, and 75% for control ewes and those receiving a CIDR on either d 0 or d 8 of the estrous cycle, respectively (P = 0.77). Wheaton et al. (1992) also observed no adverse effect on lambing percentage after CIDR removal in Columbia and Finn crossbred ewes. These authors observed a 91% lambing rate after a 30-d breeding period. Likewise, Dixon et al. (2006) reported similar conception rates in control ewes and those treated with a CIDR for 12 d. However, Foote and Waite (1965) observed reduced pregnancy rates in progesterone-synchronized ewes as did Duffey (2003) in CIDR-treated ewes. Duffey (2003) suggested that the reduced pregnancy rates may have resulted from premature demise of the CL in ewes that received a CIDR on d 0 of the estrous cycle.

IMPLICATIONS

A progesterone-containing inravaginal insert effectively synchronized estrus in mature ewes regardless of whether it was inserted on the day of estrus or day 8 of an estrous cycle. Likewise, pregnancy rate at the first estrus was not adversely impacted by the synchronization regimen.

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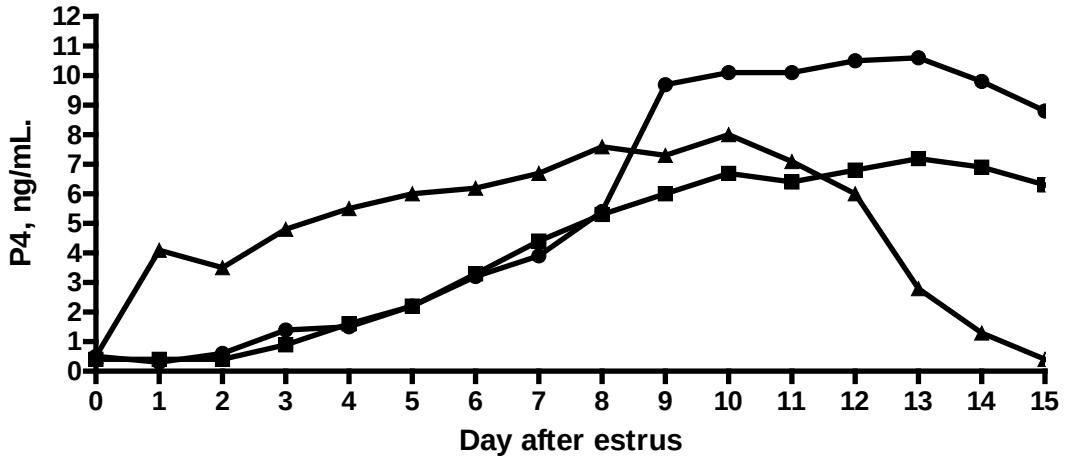

Figure 1. Serum progesterone (P4) profiles in control ewes ( ) and those receiving a P4-impregnated intravaginal insert (CIDR) on either d 0 ( ) or d 8 (luteal phase,) of an estrous cycle. The SE ranged from 0.1 to 0.6 ng/mL.

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SERUM PROGESTERONE, PROLACTIN, AND THYROID HORMONE PROFILES IN NULLIPAROUS RAMBOUILLET EWES AFTER REMOVAL OF AN INTRAVAGINAL PROGESTERONE INSERT

A. D. Stapp, K. A. Jurado, L. E. Camacho, A. N. Iroz, and D. M. Hallford
New Mexico State University, Las Cruces

ABSTRACT: Twenty-two nulliparous Rambouillet ewes (18 mo old, 59.1 ± 1.4 kg) were used to examine serum hormone profiles near the time of breeding after a synchronized estrus. Ewes were maintained in an outdoor pen (8 x 18 m) and fed alfalfa hay (1.8 kg/ewe daily) with access to water, salt, and shade. In early October, ewes received an intravaginal insert (CIDR; 0.3 g of progesterone, P4) to synchronize estrus. Fourteen days later, a blood sample was collected and the CIDR was removed (d 0). Additional blood samples were collected daily through d 5 and serum hormones were determined by RIA. As expected, serum P4 declined after CIDR removal in 12 ewes (54.5%). However, P4 remained elevated in the other 10 (45.4%) ewes. Serum P4 was 0.3 ng/mL compared with 4.7 (± 0.4) ng/mL, respectively, on d 1 and 0.7 and 4.6 (± 0.4) ng/mL on d 4 in ewes that had low or high P4 concentrations after CIDR removal. The 2 subsets of ewes were, therefore, categorized into low and high P4 status groups for examination of effects on serum prolactin (PRL), triiodothyronine (T3), and thyroxine (T4). A P4 status by day interaction was detected ($P < 0.003$) for all hormones. Serum PRL declined (quadratic, $P < 0.001$) after CIDR removal in both P4 status groups. Ewes in the low P4 group had more ($P < 0.04$) PRL than did those in the high P4 group on both d 1 (93 and 48 ± 14 ng/mL) and 2 (68 and 22 ± 12 ng/mL) after CIDR removal. Serum T3 in both P4 groups increased after CIDR removal and subsequently declined to low values on d 5 (quadratic, $P < 0.053$). Serum T3 was greater ($P < 0.02$) on d 2 in ewes in the low P4 group (1.04 ± 0.04 ng/mL) than in those in the high P4 group (0.89 ± 0.04 ng/mL). Serum T4 in low P4 ewes also increased initially after CIDR removal and then declined through d 5 (quadratic, $P < 0.001$) while serum T4 in the high P4 group was similar ($P = 0.13$) among days. On d 2, serum T4 was 57.4 ng/mL in the low P4 group compared with 50.4 (± 2.2) ng/mL for those in the high P4 group ($P = 0.03$). A relatively large percentage of nulliparous ewes failed to respond to CIDR removal with decreased serum P4. Serum PRL, T3, and T4 changed substantially after CIDR withdrawal suggesting a possible role for these hormones during follicular development which warrants further examination.

Key words: reproduction**, s**heep, synchronize

INTRODUCTION

Controlled internal drug release (**CIDR**) devices impregnated with progesterone (**P4**) have been shown to synchronize estrus in both cycling (Wheaton et al., 1993; Dixon et al., 2006) and anestrous (Knights et al., 2001) ewes. During the period in which the CIDR is in place, serum P4 concentrations are elevated to near luteal values then decline rapidly to baseline values after CIDR removal (Duffey et al., 2003). Schoenemann and Hallford (1982) treated ewes with intravaginal sponges containing the progestagen flurogestone acetate and showed that the CL of treated ewes regressed normally during the 12-d treatment period as evidenced by serum P4 values. Therefore, cycling ewes treated with intravaginal progesterone/progestagen inserts are able to produce mature follicles and ovulate shortly after the insert is removed and the P4- induced suppression of LH is relieved. Availability of the CIDR allows intensive study of endocrine events occurring at the time of ovulation and breeding. In a recent study from our laboratory, Camacho et al. (2008) observed an elevation in serum triiodothyronine (**T3**) concentrations 2 d after CIDR removal. The objective of the current experiment was to examine hormone profiles in nulliparous ewes near the time of breeding after a synchronized estrus.

MATERIALS AND METHODS

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

Animals. Twenty-two nulliparous Rambouillet ewes (18 mo old, 59.1 ± 1.4 kg) were maintained in an outdoor pen (8 x 18 m) under ambient conditions during a fall breeding season on the main campus at New Mexico State University. Ewes were fed alfalfa hay (1.8 kg/ewe daily) and had free access to water, salt, and shade. Before initiating the fall breeding period, ewes received a progesterone-impregnated intravaginal insert (CIDR; 0.3g P4; Pharmacia and Upjohn Pty Limited, Rydalmere NSW) to synchronize onset of estrus. The CIDR was removed after 14 d (d 0) and ewes were joined with fertile Rambouillet rams for a 5-d breeding period.

Blood Collection and Analysis. Before CIDR removal on d 0 and through d 5 blood was collected daily from ewes (jugular veinipuncture) into sterile serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) and allowed to clot at room temperature for approximately 30 min. Samples were centrifuged at 4°C for 15 min. at 1,500 x g and serum was stored frozen in plastic vials until assayed. Serum P4 (Schneider and Hallford, 1996), T3 (Wells et al., 2003), and thyroxine (**T4**; Richards et al., 1999) were quantified by solid phase RIA using components of commercial kits (Coat-A-Count, Siemens Medical Solutions Diagnostics; Los Angeles, CA). Serum prolactin was quantified by double antibody RIA as described by Spoon and Hallford, (1989). Within and

between assay CV were less than 15% for all hormone assays.

Statistical Analysis. When serum P4 was assayed, it was apparent that some ewes did not respond as expected to CIDR removal. When the CIDR was removed, 12 ewes experienced a decline in serum P4 while serum P4 remained elevated in the remaining 10 ewes. Ewes were, therefore, categorized into 1 of 2 P4 status groups. Those ewes that responded to CIDR removal with declining serum P4 were placed in the low P4 status group while those with persistently elevated serum P4 were placed in the high P4 status group. Effects of P4 status on serum hormone profiles were subsequently evaluated by repeated measures ANOVA appropriate for a split-plot design in which individual ewe was considered the experimental unit. Progesterone status effects were included in the main plot and sampling day and the day by P4 status interaction were included in the sub plot. Linear, quadratic, cubic, and quartic contrasts were used to examine hormonal responses across sampling days. Analyses were computed using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

Serum Progestrone. Serum P4 profiles in ewes in the low and high P4 status groups are presented in Figure 1. Comparisons between P4 status groups were conducted within sampling day because a P4 group by day interaction was detected (P= 0.006). As stated previously, all ewes received a P4-impregnated CIDR which was removed after 14 d. The first serum sample was collected before the CIDR was removed on d 0 at which time ewes in the low P4 status group had a serum P4 concentration of 2.2 ng/mL compared with 5.5 (± 0.3) ng/mL for those in the high P4 status group ($P < 0.001$). At 24 h after CIDR removal, serum P4 concentrations were 0.3 and 4.7 (± 0.4) ng/mL ($P < 0.001$) in the low and high P4 groups, respectively. This difference in serum P4 values was also observed 2, 3, 4, and 5 d after the CIDR were removed ($P < 0.001$). The decrease in serum P4 immediately after removal of the P4 containing insert in the low P4 status ewes was expected and similar results have been reported by our lab (Duffey et al., 2003; Gifford et al., 2003). Schoenemann and Hallford (1982) demonstrated that serum P4 declined as the CL regressed while an intravaginal progestagen pessary was in place. Likewise, the fact that serum P4 increased to 1.5 ± 0.5 ng/mL 5 d after CIDR removal in low P4 ewes shows that ovulation occurred and a CL had begun to produce P4. Conversely serum P4 in the high P4 status group did not decline during the 5 d after CIDR removal ($P = 0.87$, Figure 1). The reason for this failure of 45.4% of the 18-mo-old nulliparous ewes to respond to the CIDR is not readily apparent. We hypothesized that these ewes developed a persistent CL but we can not be certain since laparoscopy was not performed. Interestingly, Yates et al. (2009) stated that 12 of 57 mature CIDR-treated ewes failed to be marked by vasectomized rams within 48 h after CIDR removal. Regardless of the cause of the elevated P4, none of these ewes conceived during the 5-d period of ram exposure after CIDR removal.

Serum Prolactin. Serum prolactin profiles in ewes in the 2 P4 status groups are presented in Figure 2. A P4 status by sampling day interaction was detected ($P < 0.001$). Therefore, P4 status was examined within sampling day and day effects were analyzed within P4 status group. Prolactin was similar ($P = 0.16$) between P4 status groups on the day of CIDR removal (117 and 88 ± 14 ng/ mL for low and high P4 groups, respectively). However, on the day after the insert was removed, serum prolactin was 93 ng/mL in the low P4 group and 48 (± 14) ng/mL in the high P4 group ($P = 0.04$). Likewise, serum prolactin values were 68 and 22 (± 12) ng/mL 2 d after insert removal in low and high P4 ewes respectively ($P = 0.02$). Examination of prolactin profiles across days within P4 group revealed a quadratic response in both groups ($P < 0.007$). Prolactin values were greatest in both groups, declined precipitously through d 3 and then increased on d 4 and 5. These changes are particularly intriguing in the low P4 status ewes. The declining prolactin values near the time of estrus, ovulation, and conception warrant further investigation into this relationship.

Serum Thyroid Hormones. Serum thyroid hormone profiles are presented in Figure 3. Day of sampling by P4 status interactions were detected for both hormones ($P = 0.004$). On d 2 after CIDR removal, serum T3 values were 1.04 and 0.89 (± 0.04) ng/mL in low and high P4 ewes, respectively ($P = 0.02$). Likewise, serum T4 was elevated ($P = 0.03$) in low progesterone ewes (57.4 ± 2.2 ng/mL) compared with ewes in the high P4 status group (50.4 ± 2.2 ng/mL) 2 d after the insert was removed. This elevated thyroid hormone value near the likely time of ovulation in low P4 ewes is similar to results reported by Camacho et al. (2008). Within each P4 status group, both T3 and T4 responded in a quadratic fashion ($P < 0.05$) across sampling days after CIDR removal.

IMPLICATIONS

Twelve of 22 nulliparous 18-mo-old Rambouillet ewes responded to removal of an intravaginal progesterone containing insert with decreased serum progesterone concentrations indicative of a synchronized ovulation. Serum progesterone in the 10 remaining ewes stayed elevated for 5 d after insert removal and were thus not induced to a synchronized ovulation. Serum prolactin, triiodothyronine, and thyroxine were elevated near the time of ovulation in ewes that had decreased serum progesterone compared with those in which serum progesterone remained elevated. Involvement of these metabolic hormones in early reproductive responses warrants further investigation.

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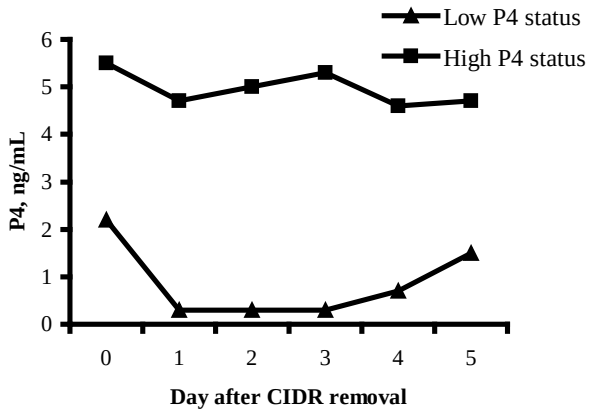

Figure 1. Serum progesterone (P4) in nulliparous Rambouillet ewes (n = 12, low P4 status; n = 10, high P4 status) near the time of breeding after removal of an intravaginal progesterone insert (CIDR). Serum P4 differed ($P < 0.001$) between groups on all sampling days. The SE ranged from 0.3 to 0.5 ng/mL.

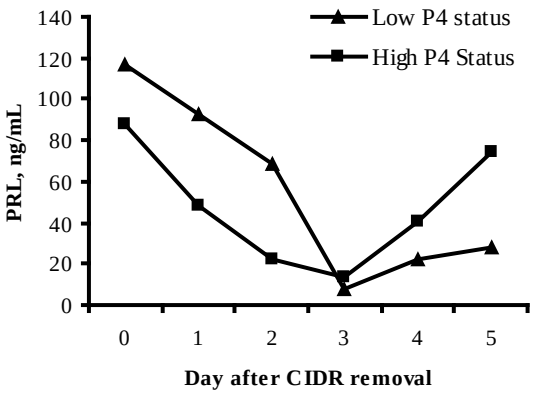

Figure 2. Serum prolactin (PRL) in nulliparous Rambouillet ewes (n = 12, low P4 status; n = 10, high P4 status) near the time of breeding after removal of an intravaginal progesterone insert (CIDR). Ewes with a low P4 status had greater ($P < 0.03$) PRL values than ewes with a high P4 status on days 1 and 2. The SE ranged from 3.0 to 17.3 ng/mL.

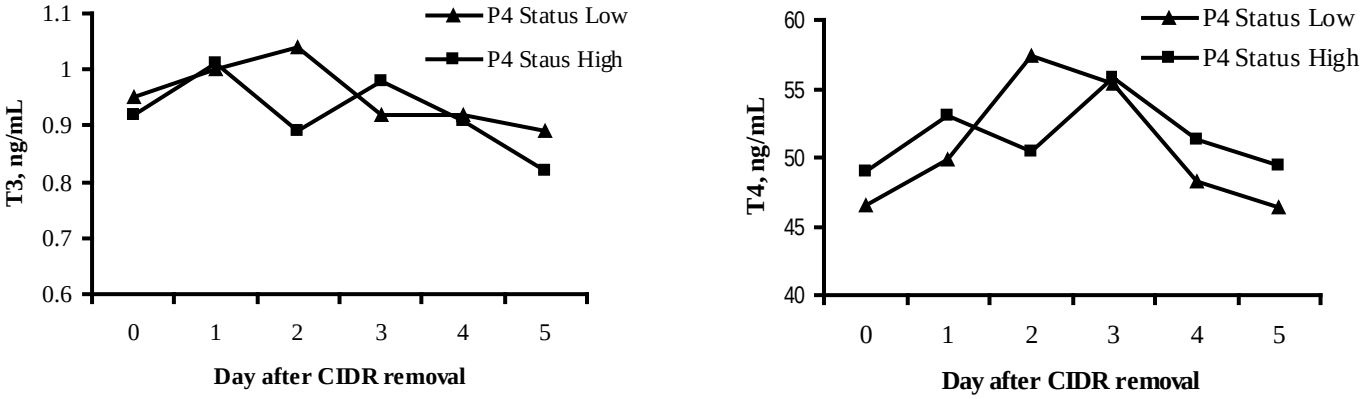

Figure 4. Serum triiodothyronine (T3, left panel) and thyroxine (T4, right panel) in nulliparous Rambouillet ewes (n = 12, low P4 status; n = 10, high P4 status) near the time of breeding after removal of an intravaginal progesterone insert (CIDR). Ewes with low P4 status had greater ($P < 0.04$) serum T3 and T4 concentrations on 2 d than those with a high P4 status. The T3 SE ranged from 0.02 to 0.04 ng/mL and T4 SE ranged from 1.8 to 2.3 ng/mL.

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MICROARRAY ANALYSIS OF BOVINE GRANULOSA CELLS FROM NORMAL AND CYSTIC FOLLICLES

J. A. Grado-Ahuir*[2], P. Y. Aad[1], and L. J. Spicer[1]
Oklahoma State University, Stillwater, OK [1];Universidad Autonoma de Chihuahua, Chihuahua, Mexico [2]

ABSTRACT: Normal ovarian follicular growth and development is regulated by extraovarian and intraovarian factors. Although ovarian follicular cysts are one common cause of reproductive failure in dairy cattle, little is known about the molecular mechanisms underlying cyst formation. Gene expression comparison may aid in elucidation of causes of ovarian cyst disease. Our objective was to identify differentially expressed genes in ovarian granulosa cells between normal dominant and cystic follicles in cattle. Granulosa cells and follicular fluid were isolated from dominant and cystic follicles collected via ultrasound guided aspiration from dairy cows (n=10 and 14, respectively), and via needle aspiration from slaughterhouse ovaries harvested from beef cows (n=13 and 10, respectively). RNA was extracted and hybridized to six Affymetrix GeneChip Bovine Genome Arrays, (Affymetrix, Santa Clara, CA). This array is designed to monitor expression of approximately 23,000 bovine transcripts through 24,072 probe sets. Affimetrix GeneChip Operating Software (GCOS version 1.1.1, Affymetrix) was used to quantitate each GeneChip. The summary intensities for each probe were loaded into DNA-Chip Analyzer (dChip), version 1.3 for normalization, standardization, and analysis. Abundance of mRNA for differentially expressed genes was determined through multiplex assays of one-step real-time RT-PCR. A total of 163 gene sequences were differentially expressed ($P<0.01$), with 19 up-regulated and 144 down-regulated and a range in fold change from 20.30 to -3.05. From selected target genes, RT-PCR analysis confirmed ($P<0.05$) angiogenin and G-protein coupled receptor 34 (GPCR34) as up-regulated in cystic follicles with 1.64- and 1.43-fold change, respectively. Because angiogenin is a potent inducer of angiogenesis, blood vessel formation may be one mechanism of cystic follicle development. These studies implicate angiogenin and GPCR34 in the development of follicular cyst formation, and identify other potential novel regulators for future study.

Key words: Cystic follicles, Microarray analysis, Granulosa cells

Introduction

Reproductive efficiency is one of the main factors affecting profitability in both beef and dairy production. Resumption of ovarian activity of dairy cows plays an important role in subsequent fertility. Among the reproductive disorders that lead to ovulation failure, follicular cysts are considered major contributors, as approximately 18 to 29% of dairy cows develop ovarian follicular cysts in a given lactation (Silvia et al., 2005). Cystic follicles are the result of some as yet undefined malfunction in the ovulatory mechanism, and since ovulation culminates after a complex series of interrelated events involving the ovary, hypothalamus, and pituitary, determining the point at which a malfunction exists is quite difficult (Eyestone and Ax, 1984). Gene expression comparisons may aid in understanding additional causes of ovarian follicular cysts, and will be vital to understand the entire process of ovulation failure and cyst formation. Thus, our objectives were to determine which genes are differentially expressed between cystic follicles and normal dominant follicles in cattle, and then establish specific hypotheses regarding regulation of the detected genes.

Materials and Methods

Animals and Sample Collection. All animal procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (IACUC protocol No. AG065). Beginning after two weeks postpartum, Holstein cows from the Oklahoma State University Dairy Cattle Center were monitored every other day via transrectal ultrasonography using an Aloka 500V ultrasound scanner with a 7.5 MHz probe. A follicle was considered cystic when it persisted for 10 or more days, with a diameter greater than or equal to 22 mm in the absence of structures that had ecogenic characteristics similar to luteal tissue and both ovaries free of corpora lutea (Silvia et al., 2005; Vanholder et al., 2006). When a cow was identified as cystic, a herdmate matched for equivalent days in milk was also monitored. The first day that the growing cystic follicle (cystic cows) or the dominant follicle (normal cycling cows) grew less than 1 mm in diameter, collection of follicular fluid and granulosa cells was performed via transvaginal aspiration as described by Santiago et al. (2005). Granulosa cells were obtained immediately by centrifugation (200 x g for 5 min) of the follicular fluid, placed in TRIzol reagent (Life Technologies Inc., Gaithersburg, MD), and stored in cryotubes at -80 °C until RNA extraction. A total of 24 follicles were aspirated transvaginally (14 cystic and 10 dominant). In addition, ovaries of beef cattle obtained at slaughter from a nearby abattoir were rinsed in ice-cold saline (0.9% NaCl), placed in a bag containing ice-cold saline-penicillin at 2500 IU and 5 mg of streptomycin per liter of solution and brought to the laboratory (1 h). Follicles present in each ovary were measured using a calipers and classified based on surface diameter, follicular fluid from cystic (25 mm or greater, ovaries lacking corpora lutea) and ovulatory follicles (15 to 20 mm) was aspirated using 20 gauge needles and 10 cm^3 syringes and centrifuged at 200 x g for 5 min at 4°C to

isolate granulosa cells. RNA was obtained and stored in the same way as samples collected by ultrasonography. A total of 23 follicles were aspirated from slaughterhouse ovaries (10 cystic and 13 dominant).

Microarray Analysis. Based on RNA concentration after extraction; six samples were selected in order to carry out a microarray experiment using GeneChip Bovine Genome Arrays (Affymetrix, Santa Clara, CA). This particular array is designed to monitor expression of approximately 23,000 bovine transcripts through 24,072 probe sets. Due to the amount of RNA required for microarrays hybridization and considering saving enough RNA for further validation of gene expression using RT-PCR, only samples from slaughterhouse harvested ovaries were used for the microarrays. Two experimental groups were formed with three samples in each group: control and cystic. Each sample was hybridized to one GeneChip. The processing of the RNA and hybridization of microarray slides were performed by the University of Tulsa Microarray Core Facility. Affimetrix GeneChip Operating Software (GCOS version 1.1.1, Affymetrix) was used to quantitate each GeneChip. The summary intensities for each probe were loaded into DNA-Chip Analyzer (dChip), version 1.3 for normalization, standardization, and analysis.

Statistical Comparison. A comparison of the mean logarithm base 2 transformed expression level of samples in the two experimental groups was performed using dChip. A critical P value of 0.01 was considered as the criteria to select a significant fold change in gene expression. This led to a final result of 163 significant probe sets.

Functional Annotation. Besides annotation produced along with statistical comparisons in dChip, the Database for Annotation, Visualization, and Integrated Discovery (DAVID, Dennis et al., 2003) as well as the NetAffx™ Analysis Center interface from Affymetrix website were utilized to correlate GeneChip array results with array design and annotation information.

Target Gene Selection. Based on novelty and lack of any previous reports in cystic follicles, six target genes were selected, three detected as up-regulated: G-protein coupled receptor 34 (**GPCR34**), angiogenin (**ANG)**, and prostaglandin E2 receptor 4 (**PGER4**); and three down-regulated: fibroblast growth factor 9 (**FGF9**), Indian hedgehog protein precursor (**IHH**), and secreted frizzled-related protein 4 precursor (**SFRP4**). Level of expression of these genes mRNA was measured in samples classified as cystic and control from both, transvaginal aspiration and slaughterhouse harvested ovaries.

RT-PCR Primer and Fluorescent Probe Design. Primers and fluorescent probes for quantitative RT-PCR were designed using Primer Express™ software (Applied Biosystems Inc.) and the PrimerQuest℠ interface of Integrated DNA Technologies, Inc. (Coralville, IA). Target gene sequences from Affymetrix were used to design the oligonucleotides (Table 1). Furthermore, a search to ensure the specificity of the designed primers and probes was performed using the Basic Local Alignment Search Tool (BLAST) interface from the National Center for Biotechnology Information (NCBI) web site (http://www.ncbi.nlm.nih.gov/sites/entrez).

One-step RT-PCR. . Differential expression was quantified following the one-step, real-time RT-PCR reaction for Taqman Gold RT-PCR Kit (Applied Biosystems Inc., Foster City, CA). Ribosomal 18S rRNA control kit (Applied Biosystems Inc.) was used as a housekeeping gene to normalize samples for any variation in RNA loading (Voge et al., 2004).

Quantification of Target gene Expression. Relative quantification of target gene expression was evaluated using the comparative threshold cycle method (Livak and Schmittgen, 2001). Abundance of mRNA was estimated setting an arbitrary threshold (CT) on the FAM or VIC curves in the geometric portion of the RT-PCR amplification plot after examining the log view. Then, the ΔCT was determined by subtracting the 18S rRNA CT from the target gene CT value. Next, for each target gene the ΔΔCT was determined by subtracting the highest ΔCT (the least expressed unknown) from all other ΔCT values. Fold changes in mRNA levels were calculated as being equal to $2^{-\Delta\Delta CT}$.

Statistical Analysis. Fold changes from the comparative threshold cycle method were log transformed [log (x+1)], and then analyzed using the MIXED procedure (SAS, 2003) under a statistical model including the fixed effect of group (cystic or control), and sample as random effect. Least squares means and standard errors were calculated through the LSMEANS sentence of the MIXED procedure. To compare the expression patterns determined through quantitative RT-PCR with that determined using microarray analysis, the ratio of the greatest to the lowest treatment least squares mean for transformed fold changes was obtained. If the mean for the control group was the highest, then a negative value was assigned to the ratio. Conversely, a positive value was assigned to the ratio if the mean from the cystic group was the highest.

Results

Analysis of hybridized GeneChip Bovine Genome arrays allowed to detect significant gene sequences ($P<0.01$) ranging from 20.30 to -3.05 fold change, with 19 elements up-regulated and 144 down regulated between control and cystic follicles. The expression patterns for target genes detected as up-regulated in the microarray analysis were consistent with the quantitative analysis (Figure 1). However, the relative level of expression estimated through RT-PCR was different ($P<0.05$) between cystic and noncystic follicles only for **ANG** and **GPCR34** (Table 2). None of the down-regulated target genes showed differences ($P>0.05$) between cystic and noncystic follicles in the relative level of expression in the quantitative analysis using RT-PCR (Table 2).

Discussion

In the bovine ovary, both protein and mRNA encoding **ANG** have previously been detected in the oocyte and in granulosa and theca cells in secondary and tertiary follicles (Lee et al., 1999). The ovary is one of the unique sites where the growth, maturation and degeneration of

blood vessels occur in a physiological state during the reproductive cycle. Regulation of the process of angiogenesis in ovarian structures involve endothelial cell-specific factors that may act alone or in concert, and whose aberrant production may lead to vascular dysfunction and ovarian disorders (Hazzard and Stouffer, 2000). **ANG** is considered a normal constituent of the circulatory system that rarely undergoes changes, but in some physiological and pathological conditions its levels increase in blood, promoting neovascularization (Tello-Montoliu et al., 2006).

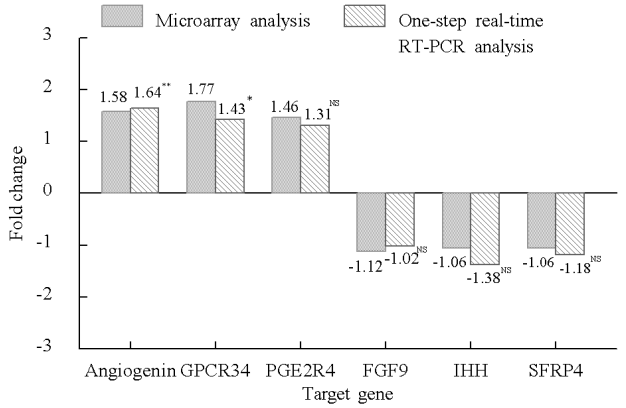

Figure 1. Comparison of fold changes in mRNA expression detected through microarray and one-step real-time RT-PCR analysis of RNA obtained from granulosa cells of cystic and noncystic ovarian follicles. All fold changes from the microarray analysis are statistically significant ($P<0.01$). Statistical differences between cystic and control follicles in the RT-PCR analysis are indicated as: N.S., not significant ($P>0.05$); * ($P<0.05$); ** ($P<0.01$).

A transcript with strong homology to **GPCR34** was detected to be up-regulated in both, the microarray and the real-time RT-PCR assay. Sugo et al. (2006) identified **GPCR34** as a receptor for lyso-phosphatidylserine, whose binding results in activation of a Gi/o-type G-protein, with consequent reduction in cAMP production and activation of phospholipase A2 (PLA2) in stimulated cells. Several phospholipases are thought to be involved in the production of lyso-phosphatidylserine (Aoki et al., 2002), which suggest that **GPCR34** may be activated in damaged and inflamed tissues. Isobe and Yoshimura (2000) described an altered balance between apoptosis and cell proliferation in granulosa and theca cells of cystic follicles. Tissue damage in the cystic follicle may translate activation of **GPCR34** to a reduction in cAMP production, which plays a crucial role in gonadotropin regulation of granulosa cells during follicle development and maturation (Conti, 2002).

Implications

Our findings of increased expression of **ANG** in cystic follicles may indicate that an increased vascularity is part of the mechanisms by which the cystic follicle is able to prolong its lifespan. Also, disruption of gonadotropin-induced cAMP signaling through **GPCR34** increased expression may impair follicle development, which could be related to the cystic condition. However, further research is required to elucidate the specific role of angiogenin and its relationship with other molecules in the cystic condition. In addition, elucidation of the importance of **GPCR34** signal cascade in regulation of cAMP and other second messengers involved in gonadotropin and growth factor stimuli on granulosa and theca cells during follicular development and cyst formation is required.

Table 2. Least squares means (LSM ± standard error) for the natural log of the relative abundance of mRNA for up- and down-regulated genes evaluated using RT-PCR in granulosa cells from cystic and noncystic follicles.

Target Gene	Group	
	Cystic	Noncystic
ANG	5.48±0.53[a]	3.34±0.46[b]
GPCR34	3.44±0.35[a]	2.40±0.30[b]
PGER4	3.30±0.42	2.52±0.36
FGF9	2.46±0.51	2.51±0.42
IHH	4.49±0.83	6.20±0.54
SFRP4	3.73±0.79	4.43±0.56

[ab] Relative abundance mean values within target gene without a common superscript are different ($P<0.05$), n=15 per mean.

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Table 1. Sequences and characteristics for primers (Forward and Reverse) and probes for real-time RT-PCR amplification of target genes.

Target gene	Sequences (forward/reverse/probe)[a]	Melting temperature (°C)	Amplicon size (bp)
GPCR34	GCTCAGGTCTTCCTGAAGTT	54	153
	CTATGTTAACAGCATTAGCCTAACA	53	
	AGAGTAAGCTCACAGTCATCACTGGAG T	61	
ANG	CTGCTACCAGAGCAAATCTACC	55	87
	CTAGTCTTGTAGGCACAGTTGG	55	
	TGCCGCGAGACAGGCAGCTCTAAGTA	65	
PGER4	CTTGGGCATGTAAGAATCATC	51	98
	CATGCTCAGTATTTCTTTGAG	49	
	CAGTCATGCTGTACACATATCTGAAGCACC	61	
FGF9	CCCTTTGCTGCTGCTAATATGTG	56	117
	CTAGAAACGTGATCCTTCCCTG	55	
	TGGCTGAGAAACACCAAGCCTCTG	62	
IHH	CGGCTTCGACTGGGTGTATTAC	59	97
	AGGGAAGCAGCCACCTGTCT	60	
	CAAGGCCCACGTGCATTGCTCC	69	
SFRP4	CTCCAAAGAGCACAAACCCA	56	100
	TAACAGGCTGTCTGGGTGAT	56	
	ACTTCCTCTCAGAGTGAGGCCAGG	63	

[a] Forward and reverse primers, and fluorescent probe for each target gene.

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SERUM HORMONE PROFILES AND CYCLIC ACTIVITY IN PREPUBERTAL RAMBOUILLET EWES TREATED WITH PROGESTERONE AND PREGNANT MARE'S SERUM GONADOTROPIN

K. A. Jurado, A. D. Stapp, L. E. Camacho, A. N. Iroz, and D. M. Hallford
New Mexico State University, Las Cruces, NM 88003

ABSTRACT: Twenty-two spring-born prepubertal Rambouillet ewe lambs (7mo of age, 44.5 ± 1.2 kg) were used to examine serum hormone profiles after progesterone (P4) and PMSG treatment. Ewes were maintained in an outdoor pen (4 x 18 m) under ambient conditions and fed alfalfa hay (1.2 kg/ewe daily) and cracked corn (0.45 kg/ewe daily) with free access to water, salt, and shade. On October 3, 11 ewes received an intravaginal P4-impregnated insert (CIDR, 0.3g P4). The CIDR was removed after12 d (d 0) and the same 11 ewes then received 400 IU PMSG (i.m.) while 11 control ewes (no CIDR) were treated with saline (i.m.). Serum samples were collected twice weekly for 4 wk before treatments were imposed, daily for the 12 d of CIDR and for 7 d after CIDR removal, and twice weekly for 6 wk after CIDR removal. Serum P4 was quantified (RIA) in all samples and serum prolactin (PRL) and insulin (INS) were measured in samples collected after CIDR removal. Before treatment began, serum P4 was less than 1.0 ng/mL indicating that ewes were prepubertal. As expected, serum P4 values increased to 4.7 ± 0.2 ng/mL on the day after CIDR insertion compared with 0.2 ng/mL in controls ($P < 0.001$). Serum P4 remained elevated for the 12-d implant period and then declined to baseline levels within 6 h after CIDR removal. Four of the 11 control ewes reached puberty during the CIDR-treatment period as evidenced by increased P4 concentrations. From 5 to 16 d after PMSG treatment, treated ewes had elevated ($P < 0.02$) serum P4 compared with controls. Serum P4 peaked at 18.4 ± 1.8 ng/mL 13 d after PMSG compared with 0.5 ng/mL on that same day in controls ($P < 0.001$). From 20 d after PMSG to the end of the study, however, serum P4 in treated ewes remained below 1.0 ng/mL while control ewes continued to have cyclic P4 profiles. Serum INS concentrations in treated ewes was 1.37 ng/mL compared with 0.64 (± 0.08) ng/mL in control ewes 2 d after CIDR removal and PMSG administration ($P < 0.001$). Likewise, serum PRL was elevated in treated compared with control ewes on d 2 (205 and 30 ± 30 ng/mL, respectively, $P < 0.001$). Progesterone and PMSG treatment in prepubertal Rambouillet ewes resulted in elevated P4 profiles indicative of follicular growth and luteal activity but cyclic activity was not maintained.

Key words: puberty, reproduction, sheep,

INTRODUCTION

Decreasing age at puberty would benefit sheep producers by allowing the ewe lamb to produce her first offspring at 1 yr of age. Females that lamb as a yearling are more productive throughout their lifetime (Hulet et al., 1969). The silent ovulation observed before the onset of cyclic pubertal activity has been hypothesized to be due to the absence of a prior luteal phase. Caraty and Skinner (1999) found progesterone (**P4**) priming to be essential for full expression of positive feedback mechanism of estradiol action on inducing the preovulatory GnRH secretion in the ewe. In certain breeds, out of season estrus can be induced by a P4-releasing device (Wheaton et al., 2003). Benavidez et al. (2007) found that administering a P4-releasing device to prepubertal ewe lambs failed to induce puberty.

Administration of PMSG immediately after removal of a P4-releasing device has been successful in allowing breeding of ewe lambs (Ainsworth and Shrestha, 1985; O'Doherty and Crosby, 1990). The PMSG produces a high degree of estrous activity and superovulatory responses necessary for prepubertal or out-of-season breeding. However, decreased fertility and variable superovulatory responses within and between breeds are common in ewe lambs treated with a combination of PMSG and a P4-releasing device. Likewise, 20 to 40% of mated ewe lambs commonly fail to lamb (Quirke et al., 1981; Ainsworth and Shrestha, 1985). Therefore, the objective of this study was to examine serum hormone profiles of prepubertal Rambouillet ewe lambs after combined P4 and PMSG treatment.

MATERIALS AND METHODS

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

Animals and Treatment

Twenty-two prepubertal spring-born Rambouillet ewe lambs (7mo of age, 44.5 ± 1.2 kg) were used to examine serum hormone profiles after P4 and PMSG treatment. Ewes were maintained in an outdoor pen (4 x 18 m) under ambient conditions and fed alfalfa hay (1.2 kg/ewe daily) and cracked corn (0.45 kg/ewe daily) with free access to water, salt, and shade. Ewe lambs were stratified by BW and randomly assigned to 1 of 2 treatment groups. Before treatment began, serum P4 was less than 1.0 ng/mL indicating that ewes were prepubertal. On October 3, 11 ewes received an intravaginal P4-impregnated insert (**CIDR**, 0.3g P4; Pharmacia and Upjohn Pty Limited, Rydalmere NSW) while controls received no CIDR. The CIDR was removed after 12 d (d 0) and the same 11 ewes then received 400 IU PMSG (i.m.; ProSpec-Tanny Techno

Gene, Ltd, Rehovot, Israel; HOR-272) while 11 control ewes (no CIDR) were treated with saline (i.m.). Body weight measurements were taken every 21 d throughout the experiment.

Blood Collection and Analysis

Blood was collected (jugular venipuncture) from ewe lambs twice weekly for 4 wk before treatments were imposed, daily for the 12 d of CIDR and for 7 d after CIDR removal, and twice weekly for 6 wk after CIDR removal. Blood was collected into sterile serum separator tubes (Corvac, Kendall Health Care, St. Louis, MO) and allowed to clot at room temperature for approximately 30 min. Samples were centrifuged at 4°C for 15 min at 1,500 x g and serum was stored frozen in plastic vials until assayed. Serum P4 was determined in all samples and prolactin and insulin were determined in samples collected after CIDR removal. Serum P4 (Schneider and Hallford, 1996) and insulin (Reimers et al., 1982) were quantified by solid phase RIA using components of commercial kits (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA). Serum prolactin values were determined by double antibody RIA as described by Spoon and Hallford (1989). Within and between assays CV were less than 15% for all hormones. Puberty was determined by serum P4 concentrations greater than 1ng/mL for 3 or more consecutive days.

Statistical Analysis

Effects of treatment on ewe lamb BW were examined by ANOVA for a completely random design. Analyses of BW were computed using the GLM procedure of SAS (SAS Inst. Inc, Cary, NC). Serum hormone responses to treatment were subjected to repeated measures ANOVA for a split-plot design. Treatment effects were included in the main plot and treatment by sampling day interactions were included in the sub plot. Analyses of hormone profiles were computed using the mixed procedure of SAS.

RESULTS AND DISCUSSION

Ewe Body Weight

Before CIDR treatment, ewe lambs averaged 44.5 ± 1.2 kg. After 21 d control ewes weighed 46.8 ± 1.9 kg compared with 44.7 ± 1.9 kg for CIDR-PMSG-treated ewe lambs ($P = 0.44$). This similarity in BW between groups continued throughout the experiment and after 56 d BW were 54.1 and 52.3 (± 2.2) kg for control and treated ewes, respectively ($P = 0.53$). These data demonstrate no adverse effect of CIDR and PMSG on BW responses of ewe lambs.

Serum Hormone Profiles

Serum Progesterone. Before treatment began, serum P4 was less than 1.0 ng/mL in all ewes indicating that they were prepubertal. Serum P4 profiles during the 12-d period in which CIDR were in place are shown in Figure 1. A treatment by sampling day interaction was detected ($P < 0.001$) necessitating examination of CIDR treatment effects on P4 within day. On the day after CIDR insertion, serum P4 values were 4.7 ± 0.2 ng/mL compared with 0.2 ± 0.2 ng/mL in controls ($P < 0.001$). On d 6, serum P4 concentrations were 1.0 and 4.8 (± 0.4) ng/mL in control and CIDR-treated ewes, respectively ($P < 0.001$). Four of the 11 control ewes reached puberty during the CIDR treatment period as evidenced by increased P4 concentrations. This response resulted in average P4 values for control and CIDR-treated ewes of 1.7 and 2.9 (± 0.7) ng/mL, respectively, on d 12 ($P = 0.22$). These data demonstrate that CIDR effectively raised serum P4 concentrations. Benavidez et al. (2007) reported a similar response.

During the 4-d period immediately after CIDR removal, serum P4 averaged 1.5 and 0.9 (± 0.6) ng/mL in control and treated ewes, respectively ($P = 0.46$) demonstrating the rapid decline in serum P4 after the insert was removed. As mentioned previously, CIDR-treated ewe lambs also received PMSG on the day of CIDR removal. Serum P4 profiles from 5 to 27 d after insert removal and PMSG treatment are shown in Figure 2. Treated ewes had elevated ($P < 0.02$) serum P4 compared with controls from 5 to 16 d after PMSG treatment (treatment by day, $P < 0.001$). Serum P4 peaked at 18.4 ± 1.8 ng/mL 13 d after PMSG compared with 0.5 ng/mL on that same day in the controls ($P < 0.001$). From 20 d after PMSG to the end of the study, however, serum P4 in treated ewes remained below 1.0 ng/mL while control ewes continued to have cyclic P4 profiles. These data indicate that PMSG resulted in elevated serum P4 indicative of luteal activity suggesting that follicular growth and ovulation was induced. However, verification of this awaits quantification of serum estradiol.

Serum Insulin. Insulin was measured in samples collected on the day of CIDR removal and PMSG administration and for 6 d thereafter and concentrations are presented in Figure 3. Insulin values on both the day of insert removal and the day after were similar ($P > 0.25$) in control and treated ewes (treatment by day, $P = 0.006$). However, 2 d after PMSG treatment, insulin in control ewes was 0.64 ± 0.08 ng/mL compared with 1.37 ± 0.08 ng/mL in treated ewes ($P < 0.001$). From 3 to 6 d after PMSG administration, serum insulin did not differ between treatments ($P > 0.14$). Downing et al. (1995) suggested that an increase in insulin-mediated glucose uptake by follicles could be the stimulus responsible for an increase in ovulation rate. The increase in insulin we observed 2 d after PMSG treatment could be attributed to this response.

Serum Prolactin. Figure 4 presents prolactin profiles beginning on the day of CIDR removal and PMSG administration and continuing for 6 d thereafter. A treatment by day interaction was detected ($P = 0.01$) necessitating comparison of treatment effects within day. Prolactin concentrations were similar ($P = 0.43$) between treatments on the day of PMSG administration. On the day after treatment ewes receiving PMSG tended ($P = 0.08$) to have greater serum prolactin values (142 ± 38 ng/mL) than did the controls (41 ± 38 ng/mL). Two days after treatment, serum prolactin in control ewes was 30 ng/mL while PMSG-treated ewes had a prolactin concentration of 205 ± 30 ng/mL ($P < 0.001$). Serum prolactin was also greater in treated than in control ewes 5 and 6 d ($P < 0.08$) after PMSG administration. These data demonstrate an elevation in prolactin during the period of follicular development and ovulation in ewe lambs treated with P4 followed by PMSG

after removal of the P4. This possible involvement of prolactin in induced ovulation in prepubertal ewe lambs warrants further investigation.

IMPLICATIONS

Treatment of 7-mo-old spring born ewe lambs with intravaginial progesterone followed by PMSG resulted in serum progesterone profiles indicative of luteal activity. Subsequent studies should evaluate estrous and mating responses following use of this treatment regimen.

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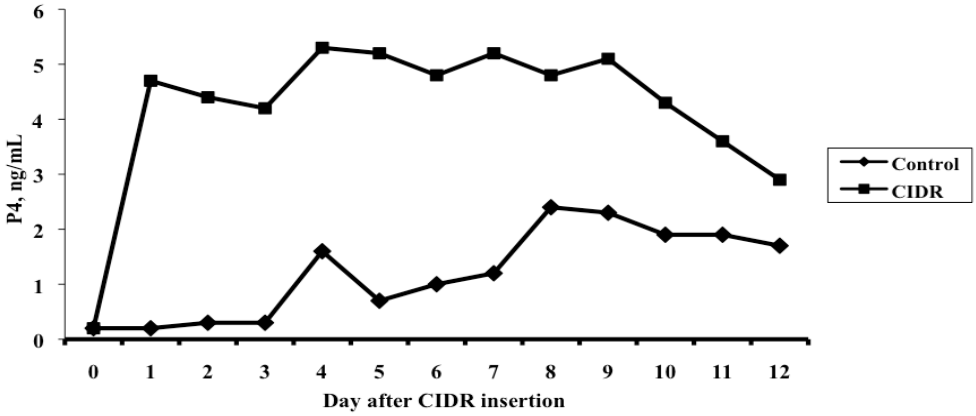

Figure 1. Serum progesterone (P4) in 7-mo-old spring born ewe lambs during a 12-d period in which a progesterone-impregnated intravaginal insert (CIDR) was in place (d 0 = day of CIDR insertion). Serum P4 values differed ($P < 0.05$) on d1 through 11 (treatment by day, $P < 0.001$). The SE ranged from 0.1 to 0.7 ng/mL.

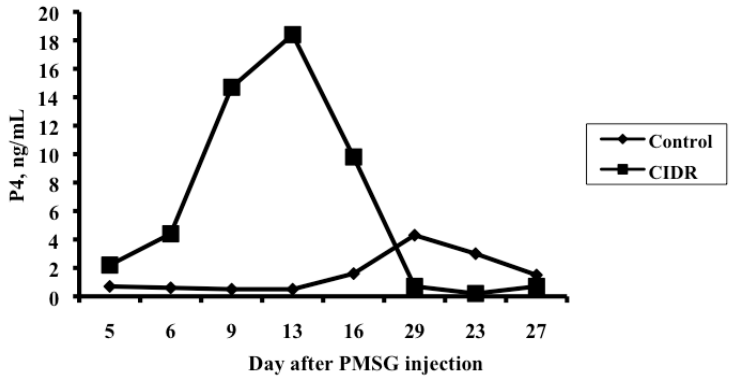

Figure 2. Serum progesterone (P4) in 7-mo-old spring born ewe lambs after PMSG injection (400 IU i.m.; immediately after progesterone-impregnated intravaginal insert (CIDR) removal). Serum P4 values differed ($P < 0.02$) on d 5 through 16 (treatment by day, $P < 0.001$). The SE ranged from 0.4 to 2.2 ng/mL.

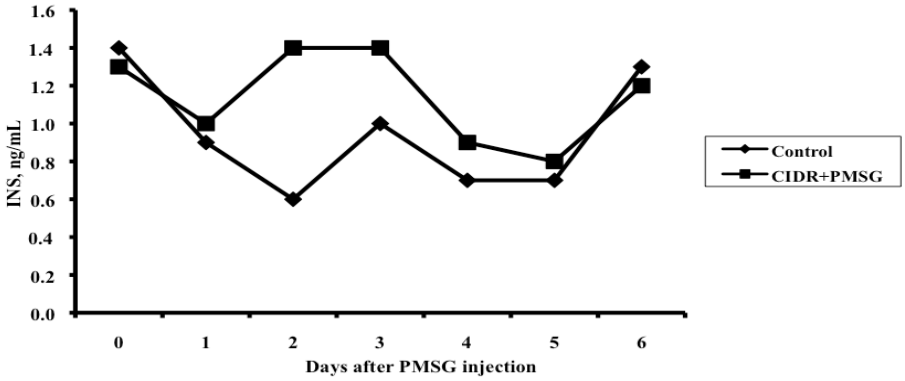

Figure 3. Serum insulin (INS) in 7-mo-old spring born ewe lambs after PMSG injection (400 IU i.m.; immediately after progesterone-impregnated intravaginal insert (CIDR) removal). Serum INS values differed ($P < 0.001$) on d 2 (treatment by day, $P < 0.006$). The SE ranged from 0.08 to 0.17 ng/mL.

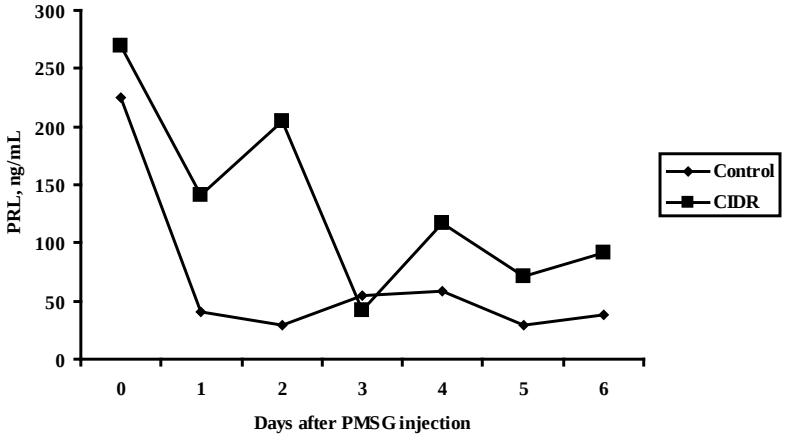

Figure 4. Serum prolactin (PRL) in 7-mo-old spring born ewe lambs after PMSG injection (400 IU i.m.; immediately after progesterone-impregnated intravaginal insert (CIDR) removal). Serum INS values differed ($P < 0.001$) on d 2 (treatment by day, $P < 0.01$). The SE ranged from 14 to 39 ng/mL.

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EFFECT OF POST WEANING GNRH-A INJECTION ON REPRODUCTIVE PERFORMANCE OF SOWS

J. A. Romo[1*], J. M. Romo [2, 1], O. S. Acuña[1], H. R. Guemez[1, 2], R. Barajas [1], F. Juarez[1], and S. A. Felix[1]
[1]Facultad de Medicina Veterinaria y Zootecnia de la Universidad Universidad Autonoma de Sinaloa
[2]Granja Porcina "La Huerta"
E-mail: romo60@uas.uasnet.mx

ABSTRACT: With the objective of determine the effect of after weaning GnRH-A injection on reproductive performance of sows, in a completely randomized design experiment 92 hybrid (York Shire, Landrace, Hamp Shire, Duroc, and Pietrain) sows were used. Treatment tested were: 1) Sows receiving injection of 1 mL of saline solution at 24 and 72 hours after weaning (Ctrl; n = 41); or 2) Sows receiving 50 µg of GnRH-A at 24 and 72 hours after weaning (GnRH; n = 51). GnRH-A application decreased (P = .07) weaning-to-estrus interval (7 ±4.7 vs. 5.6 ±2.6 days); and improved (P < .01) estrous rate inner first seven days after weaning (80 vs. 96%); and trend to enhance (P = .14) first service parturition rate after weaning (88 vs. 94%). Litter size tended (P = .12) to be increased on the control group sows, and litter weight at born was not affected by treatments (P > .20). Results indicates that injection of 50 µg of GnRH-A at 24 and 72 hours after weaning is a available tool to reduce weaning to estrus interval of the sows, improves number of sows that return to cyclic activity inner first seven days after weaning, and increases farrowing rate of sows at first service after weaning.

Key Words: GnRH, Reproductive performance, Sows.

Introduction

Restarting cyclic activity of the sows after weaning is key on its subsequent reproductive performance. Estrus is associated with the presence of larges and healthy follicles selected during proestrus and undergoing terminal maturation (Sirois and Fortune, 1988). During this period, concentrations of progesterone decrease and the large follicles produces 17b-estradiol that is involved in triggering estrus via its positive feedback on the hypothalamus-hypophysis center (Ireland, 1987; Fortune et al., 1988).

This stimulates GnRH-induced release of luteinizing hormone (LH) pulses and the LH surge, which is ultimately responsible for ovulation of the large follicle (Clarke, 1987; Nett, 1987). Failure to return to estrus in swine may be due, at least in part, to an increased sensitivity of the hypothalamo-hypophyseal axis to the negative feedback effect of estradiol (Almond and Dial, 1990). In this sense, has been observed that an increase in serum estrogens preceded the preovulatory surge LH by about 48 h, both during the estrous cycle (Henricks et al., 1972) and at the after weaning estrus (Stevenson et al., 1981). This increment is dependent of the numbers preovulatory follicle presents after weaning in the sows. The GnRH and its agonists/analogues operate on ovarian follicular development and CL function indirectly via the induced release of pituitary LH and FSH (Conn and Crowley, 1994). Administration of GnRH increases LH and FSH in the peripheral circulation within 2 to 4 h (Chenault et al., 1990; Rettmer et al., 1992; Stevenson et al., 1993). These gonadotropins act directly by binding to their respective receptors on follicular and luteal cells. Thus, Gonadotropin releasing hormone analogues (GnRH-A) has been converted in an alternative for stimulate the growth follicular and ovulation (Hühn et al., 1996). In this sense, has been observed that estrus and ovulation are induced by GnRH-A intravenous pulsate injection in prepubertal gilts (Lutz et al., 1984), lactation period (Cox y Britt, 1982), and postpartum-anestrous sows (Britt et al., 1985), reducing the depressing effects of suckling and lactational catabolism on the gonadotropin secretion (Brüssow et al., 1996). To respect, Szabó et al. (1991; 1992) observed improves on the litter size at birth time, number of sows that inner in estrous within first seven days after weaning when applicated GnRH-A both after as before of weaning, respectively. Romo et al. (2005) observed that GnRH-A application four days before weaning improves the farrowing rate on young sows. Thus, the objective present study was to evaluate the effect of the analogous of gonadotropin releasing hormone (GnRH-A) applied twice at 24 and 72 hours after weaning on reproductive performance sows.

Material and Methods

The experiment was realized from February to December 2007, with the services of the commercial pork farm "La Huerta" localized in Culiacan, Sinaloa, in the Northwest of Mexico (24º45' N; 107º 31' W; 23 m over mean sea level). Nine two multi-parturient hybrid-sows (York Shire, Landrace, Hamp Shire, Duroc, and Pietrain) were used. In agreement to a completely randomized experiment design, sows were assigned to receive one of two treatments: : 1) Sows receiving injection of 1 mL of saline solution at 24 and 72 hours after weaning (Ctrl; n = 41); or 2) Sows receiving 50 µg of GnRH-A at 24 and 72 hours after

weaning (GnRH; n = 51). Remainder management was similar for sows placed in both treatments.
Information of weaning to estrous interval (WEI), total pig birth (TPB), pig birth alive (PBA), litter weight at birth (LWB), farrowing rate at first service after weaning (FR), and estrous presence inner first seven days after weaning (ER) were registered during one reproductive cycle.

Statistical analysis. The data of WEI, TPB, PBA, LWB, were analyzed as a Completely Randomized Experimental design (Hicks, 1973), using the ANOVA/COV procedure ANOVA/COV for generals lineal models of the program Satistix ® 8 (Analytical Software; Tallahassee, FL). Data of FR and ER were analyzed by X^2 using 2 x 2 contingence tables.

Results and Discussion

Effects of GnRH-A application on weaning to estrous interval of multi parturient sows is shown in Table 1.

Table 1. Effects of GnRH-A application at 24 and 72 h after weaning on weaning to estrous interval of multi parturient sow

Variables	Treatments		SEM[1]	P-value
	Ctrl.	GnRH		
Sows, n	41	51		
WEI, days [2]	7.0	5.6	0.39	.07

[1] Standard error of the mean, [2] weaning to estrous interval.

Influence of GnRH-A application on sows farrowing performance is offered in Table 2 and on the estrous presence inner first seven days after weaning in Table 3.

Table 2. Effects of GnRH-A application at 24 and 72 h after weaning on sows farrowing performance

Variables	Treatments	
	Ctrl.	GnRH
Sows, n	33	48
Parturient sows, n	29	45
Non parturient sows, n	4	3
Farrowing rate, % [1]	88 [a]	94 [b]

[a, b] Letters distinct in a same row indicates statistical trend, $P = 0.14$

In this study the application of 50 µg of GnRH-A at 24 and 72 hours after weaning of the sows diminished ($P < 0.07$) in 20% from 7 to 5.6 days the weaning to estrus interval; and increased ($P < 0.01$) 16% the percentage of sows that showing estrus inner seven days after weaning (80 vs. 96%). These results are in concordance with findings of Szabó et al. (1991); they observed that 96.7% of the sows coming into estrus at response to GnRH-A application 48 h after weaning, besides they found that conception rate was improved. In other study realized for Szabó et al. (1992), they observed that treatment with GnRH-A at ending of lactation period (day 19) it reduced as that as 50% the number of sows failing to come into estrus after weaning, and increased 42% the number of sows coming into estrus within one week after weaning. Cox and Britt (1982); Rojanasthien et al. (1987); and De Rensis et al. (1991) observed an increment on follicular development in response to exogenous GnRH treatment during lactation, consistent with results of actual experiment.

Table 3. Effects of GnRH-A application at 24 and 72 h after weaning on sows coming into estrous inner seven days post weaning

Items	Treatments	
	Ctrl.	GnRH
Sows, n	41	51
Sows that shown estrous inner seven days after weaning, n	33	49
Sows that shown estrous outer seven days after weaning, n	8	2
Estrous rate inner seven days after weaning, %	80[a]	96[b]

[a, b] Letters distinct in a same row indicates statistical difference, $P < .01$

Effects of GnRH-A application to sows on litter characteristics are presented in Table 4.

Table 4. . Effects of GnRH-A application at 24 and 72 h post-weaning on litter characteristics

Variables	Treatments		SEM[1]	P-value
	Ctrl.	GnRH		
Sows, n	29	45		
TPB[2], n	11.034	10.067	.3061	.12
PBA[3], n	10.172	9.111	.3080	.09
LWB[4], kg	14.397	13.493	.4509	.33

[1] Standard error of the mean, [2]total pig birth, [3]Pig birth alive
[4]litter weight at birth

The application of 50 µg of GnRH-A at 24 and 72 hours after weaning improved ($P = .14$) the farrowing rate of sows at first service after weaning. This result is similar at the observed by Szabó et al. (1992) when applied GnRH-A 48 h after weaning. Romo et al. (2005) indicated that GnRH-A application fourth days before weaning increases the farrowing rate in the young sows; they observed an increment of 15% on this variable (control group 69% vs. GnRH-A group 84%).

Results of this experiment indicate that GnRH-A application diminish the weaning to estrus interval, increase the number of sows coming into estrus one week and the farrowing rate at first service after weaning.

Implications

Application of 50 µg de GnRH-analogue at 24 and 72 h after weaning diminishes the weaning to estrus interval and increases the percentage of sows that present estrus within of the first seven days after weaning, and the farrowing rate at first service post-weaning. Then is an available tool to improve the reproductive performance of sows.

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OVARIAN ACTIVITY POSPARTUM IN HOLSTEIN COWS IN NORWEST OF MEXICO

M.P. Gallegos,[1] H.L. Castro,[1] J. R. Cervantes,[2] J.F. Villarreal,[2] *J.S. Saucedo,[3] A.P. Márquez[3], M.F. Montaño[3]
[1]Universidad Juárez del Estado de Durango, México, [2]Universidad Autónoma de Nuevo León, México, [3]Universidad Autónoma de Baja California, México

ABSTRACT. Data came from a dairy herd located at the northwest region of México. Blood serum samples (n=2830) of Holstein cows (n=113) were used. The objective was to characterize the ovarian activity postpartum of Holstein cows through the concentration of (P_4). Blood samples were processed from parturition to visual estrus at 102 d pp. Data was analyzed by using Main Components Procedure. The resumption of ovarian activity (RAC) was the response variable. The (RAC) appeared at 31 d in 77% of the cows under study. It was found that cows of highest milk yield, best condition score, and higher body weights pre and post partum that calved during summer and fall seasons shown a shorter post partum interval-(RAC). Estrous was detected visually at (56 and 68, d pp) in cows that give birth on the summer and during the other seasons, respectively. Cows shown 2 through 4 luteal phases at 102 d pp, attributable to seric levels (P_4). These results based in limited numbers suggest the presence of silent estrus cycles before showing estrus signs.

Key Words: Ovarian activity, Postpartum, Holstein cows

Introduction

Even though the advance in the knowledge about the reproductive cycle and the increment of the possibilities to manipulate it. The decrement in the fertility in the dairy herds with high production continues (Opsomer *et al*., 2006), increasing considerably the interval between partum. Sheldon *et al*., (2006) consider that each heard and each cow between herds is different, therefore it is not possible implement measures of general handling. Opsomer *et al*., (1998) says the increment in the milk production has contributed 49% of the cows do not reinitiate the ovarian-cyclical activity in the first 50d post partum, or that prolonged luteal-phases are presented, (more of 20 d). Not only the partum-insemination period was increased, also the first conception-service interval was extended in the high producer cows; as a consequence of a failed fertilization, fetal embryonic death and/or r fetal and/or problems in the estrus detection (Opsomer *et al*., 2002). It is mentioned that besides the dairy production and some common patofiosiologico process during the milky cattle pp phase, the negative energetic balance (NEB) influence in a negative way in the fertility. Lamming and Darwash (1998); Mateus *et al*., (2002), mention that the sub fertility is because of the uterine or ovarian dysfunction and an abnormal endocrine pattern, which change the cycles length, ovarian cysts or embryonic death and the pathological processes caused by concomitant opportunist microorganisms with the involution uterine which they can avoid another_conception (Kask *et al*., 2000). Actually a debate exists in relation to optimal economic point interval between partum in this type of cattle. Nevertheless, one aspect in consideration is the absence of particular information of cattle behavior related to places, handling and types of climate where they are breeding. The objective of this work was to characterize the resumption of cyclical and ovarian activity on the Holstein cattle under the estabulation conditions on a semi-arid weather.

Materials and Methods

The work was made with 113 exploited Holstein cows in a semi-arid weather with a temperature and average annual precipitation of 21 ºC and 573 mm. During measure phases the maximum and minimum temperature registered were 16.11 ± 6.21 max and 30.00 ± 5.08 ºC respectively the evaporation was 6.13 ± 2.11 % and the pluvial precipitation was 14.01 ± 19.44 mm. The animals were feed twice per day with a ration of concentrated food and seasonal forage (sorghum or corn in the summer and fall, or oaths, silo corn or sorghum during the winter and spring). As far as reproductive handling, the estrus detection was made twice per day (AM and PM) 30 minutes each time, after the 20 d pp. The cows in which estrus was present were inseminated after a gynecological revision, based in the AM/PM rule. The sanitary handling was uniform and according to the region. The mechanical milking was twice per day (4:00 and 15:00 h). The corporal condition (CC) was determined by the proposed scaled by Wildman *et al*., (1982) pre and post partum. 2830 blood serum samples were taken from coccygeal vein of each animal, since the partum until estrus was detected or the first 102 days pp, what happen first. The test period lasted 455 d the serum sanguineous was stored at -56 ºC until the determination of progesterone by solid phase RIA. To identify the causes that change the resumption of the ovarian activity 20 variables were tested; the main components were analyzed with the purpose of reduce the number and together those ones which indicate the same pattern; the effect of those components about the resumption of ovarian activity was evaluated through linear regression analysis (SPSS, 1997).

Results and Discussion

The resumption ovarian activity in average appeared to the 31 d pp (rank 11 to102 days) in 76% of the cows; the rest did not show ovarian activity until 102 d pp. The main component analysis showed that six components (variables) can explain the total variance 86% (Table 1). The component with major characteristic was “Environment conditions” (maximum and minimum temperature and

evaporation). The 2nd component "Milk production pp", the variables related with the milk production in the 1st. trimester pp was included. The 3rd component was "Thermal Variation" in which the daily oscillation of the average temperature is preponderant. The calf weight at birth and the weight diminution pp conform the 4th main component "Calf weight at birth". The 5th component was "Corporal Condition" and 6th "Weight loss pp" because of the difference between pre and post partum is the most important variable in this component (Table 1). The best model of multiple linear regression was obtained the 2nd, 3rd, 4th and 5th main extracted components, which result highly significant ($p = 0.01$) over the resumption of ovarian activity (ROA). The partum were influenced significantly by the season of the year ($p = 0.015$) over the resumption of ovarian activity , the ROA was presented earlier in the cows which gave birth in the summer and fall (27.11 ± 13.07 y 27.66 ± 15.64 d) than the cow which gave birth in winter and spring (37.65 ± 17.52 y 42.55 ± 23.20 d). The cows which gave birth in the summer their estrus were detectable at 56.20 ± 6.15 d, while the cows which gave birth in the fall, spring and winter estrus were detected at 76.81 ± 44.80; 81.43 ± 34.87 y 96.75 ± 51.05 d pp respectively. In Figure 1, it shows the time in which each luteal phase pp was presented, the length and the period between luteal phases LP in relation to he seric concentration of progesterone. The LP length was 21.7 d while the P_4 concentration in the LP before the estrus detected was 10.93 ± 10.46 ηg/mL. The results did not show that a lineal relation exist between the main components found in this study with the added P_4 concentration in the Holstein cows ($p = 0.60$). In this case the ROA is the same reported by El-Din Zain *et al*., (1995) who report a rank of 17 a 42 d and at 45 a 60 new LP were started. In this study and in agreement to the P_4 concentrations and LP length, two estrus cycles were presented between 31 a 69 d pp, but they were not detected in visual shape (Figure 1). The cows which got the most production and with higher pre and post pp weight has a minor partum ROA interval, which this agreed with Gwazdauskas *et al*., (2000), about the ROA after the 40 d pp. Senatore *et al*., (1998) also report that the heavier cows had a short interval to the 1st ovulation probably because of the resistance to the negative energetic balance. On the other side, the season of the year has influence over the ROA pp contrary to the report by EL-DIn Zain *et al*., (1995). In this study was found that the cows which gave birth in the summer RAO pp and the estrus is detected earlier than the other ones giving birth on the other seasons, all this do not agreed with Thompson *et al*., (1996). Once the ovarian activity restart, there was not difference between the length of the estrus cycle (Fig. 1); in this case Lamming and Darwash (1998) say that the estrus cycle length it should not be more than 25 days (18 days maximum of luteal phase and 7 days of interluteal interval). Also it was found that the seasons of the year with a higher thermal oscillation and evaporation, the interval between the partum-ROA was increased in disagreement with the written by EL-Din Zain *et al*., (1995).

Conclusions

An evident association was found between the estrus cycles and the observed primer-partum interval, which indicate that the cows restart their ovarian activity and even three complete estrus cycles were shown between the 102 d pp, without estrus behavior estrus were detected. The ovarian activity pp in breeding dairy cows in the Northwest Region of Mexico is positively correlated with the milk production in the first trimester pp, the weight pp and the prepartum. It had been concluded that the dairy cows which give birth in the summer and fall restart their ovarian activity earlier than the ones which give birth in the winter and spring, like the ones which give birth with a better corporal condition.

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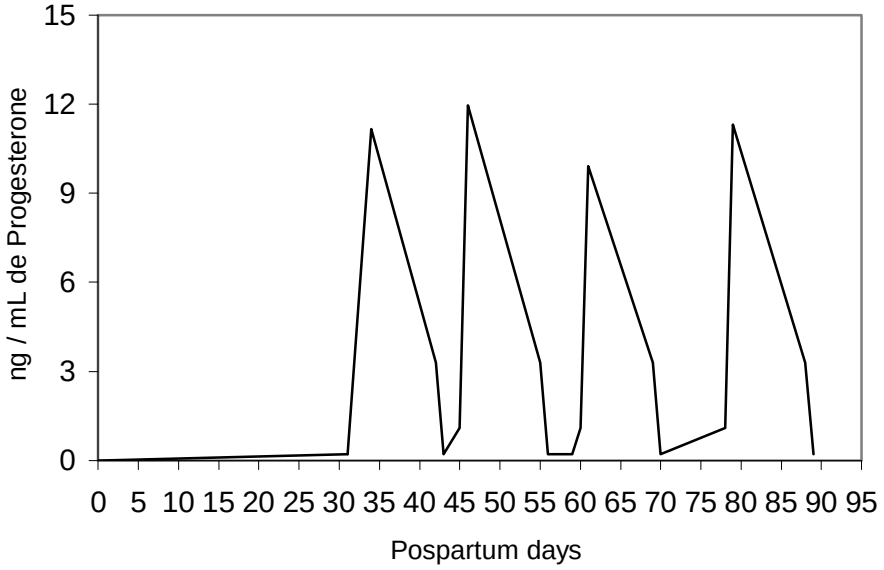
Table 1. Analysis of extraction of the main components that explain causal of variance in estimators obtained in Holstein cows

	Main components			Sum of squares considered in the extraction		
Component	Total	% of Variance	Accumulated %	Total	% of Variance	Accumulated %
1	1	5.97	29.86	29.86	5.97	29.86
2	2	4.03	20.13	49.99	4.03	20.13
3	3	2.33	11.67	61.66	2.33	11.67
4	4	1.72	8.61	70.27	1.72	8.61
5	5	1.24	6.22	76.49	1.24	6.22
6	6	1.03	5.17	81.66	1.03	5.17
7	7	0.90	4.51	86.18		
8	8	0.66	3.31	89.49		
9	9	0.50	2.50	91.98		
10	10	0.47	2.36	94.34		
11	11	0.35	1.77	96.12		
12	12	0.30	1.49	97.60		
13	13	0.22	1.08	98.69		
14	14	0.19	0.94	99.62		
15	15	0.06	0.28	99.90		
16	16	0.02	0.10	100.00		
17	17	0.00	0.00	100.00		
18	18	0.00	0.00	100.00		
19	19	0.00	0.00	100.00		
20	20	0.00	0.00	100.00		

Table 2. Start day, length of the luteal phase pp and average concentration of progesterone in Holstein cows

Oestrus cycle postpartum	n	Luteal phase Start Media ± DE (d)	Length of Luteal phase Media ± DE (d)	P_4 Concentration Media ± DE (ηg/mL)
1	87	31 ± 17.29a	11.7 ± 1.6a	11.16 ± 15.89[a]
2	58	45 ± 15.46a	9.5 ± 0.5a	11.96 ± 6.90[a]
3	21	60 ± 11.10b	9.7 ± 0.8a	9.91 ± 7.61[a]
4	14	79 ± 12.90b	9.9 ± 0.9a	11.30 ± 4.59[a]
TOTAL				10.93 ± 10.46

* Different literals in the columns indicate statistical difference (p = 0.05)



Graphic 1. Representation of the resumption of the ovarian activity and cyclical behavior reproductive post-partum in Holstein cows

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EFFECTS OF CORN CONDENSED DISTILLERS SOLUBLES SUPPLEMENTATION ON DRY MATTER INTAKE, PERFORMANCE, RATE AND SITE OF DIGESTION, AND RUMINAL FERMENTATION IN STEERS FED MODERATE QUALITY FORAGES

L. R. Coupe, G. P. Lardy, T. C. Gilbery, A. M. Meyer, J. L. Leupp, M. L. Bauer, and J. S. Caton
Department of Animal Sciences, North Dakota State University, Fargo

ABSTRACT: Five ruminally and duodenally cannulated Holstein steers (755 ± 68 kg of initial BW) were used in a 5 x 5 Latin square to evaluate the effects of corn condensed distillers solubles (CCDS; 20.4% CP, 15.6% EE, 0.1% Ca, 1.2% P, 1.2% S; DM basis) supplementation on intake, site of digestion, and ruminal fermentation in steers fed moderate-quality forage. Steers were offered ad libitum forage (8.2% CP, 73.6% NDF, 47.6% ADF, 6.5% ash; DM basis), consisting of a mixture of 40% mature bluestem hay and 60% mixed grass alfalfa hay. Steers were individually penned (3.0 x 3.7 m) during each 7-d adaptation period then placed in individual metabolism stalls during each 7-d collection period. Treatments were arranged in a 2 x 2 + 1 factorial design; main effects were CCDS feeding method (mixed vs. fed separately) and level of CCDS (0.2 vs. 0.4% BW). The resulting 5 treatments were a negative control (no supplement), 0.2% BW CCDS mixed with the forage, 0.4% BW CCDS mixed with the forage, 0.2% BW CCDS fed separately, and 0.4% BW CCDS (DM basis) fed separately. Supplementation with CCDS increased ($P = 0.04$) total DM and OM intake compared to control. Steers fed CCDS separately had increased ($P = 0.04$) total DM and OM intake compared with steers fed mixed diets. Total tract OM digestion increased ($P = 0.01$) in steers fed separate diets compared to mixed diets. Apparent and true ruminal CP digestion was increased ($P = 0.05$) in supplemented steers and those fed mixed diets. Control steers had increased ($P = 0.03$) total tract NDF and ADF digestion compared to supplemented steers. Steers fed 0.2% BW CCDS had increased ($P = 0.03$) total tract NDF and ADF digestion compared to steers fed 0.4% BW CCDS. Mixed diets decreased ($P = 0.04$) ruminal pH compared to separate diets. No treatment effects were observed for ruminal fill, fluid dilution rate, or microbial efficiency ($P \geq 0.17$). Results of this study suggest that CCDS supplementation increases intake and CP digestion but has minimal effects on fiber utilization and microbial efficiency in steers fed moderate-quality forages.

Key words: corn condensed distillers solubles, digestion, forage

Introduction

The ethanol industry is expanding throughout the Midwest, consequently producers have the option to utilize these byproducts (RFA, 2008). Corn condensed distillers solubles (CCDS) is becoming more popular as a protein supplement. Corn condensed distillers solubles are relatively high in CP and fat, which makes this product appealing for supplementing beef cows (Coupe et al., 2008).

Corn condensed distillers solubles are high in both protein and fat (15-25% CP and 4-22% fat, DM basis; Gilbery et al., 2006; Da Cruz et al., 2005). Gilbery et al. (2006) reported two studies that used CCDS for supplementing low-quality forages which gave conflicting results. In the first study, forage DMI was not affected by increasing CCDS level when CCDS was fed separately from forage. In addition, total tract ADF and NDF digestibilities were not affected by increasing level of CCDS. However, in a second study when CCDS was mixed with forage, forage DMI increased quadratically with the greatest DMI at 10% CCDS. There was also a linear increase in ADF and NDF ruminal digestion when increasing levels of CCDS were mixed with forage. One explanation for the differences between the studies could be that feeding CCDS and forage together results in improved synchrony and release of nutrients (Gilbery et al., 2006). However, more research is needed to better understand the differences in DMI and digestibility that occurs when CCDS is fed mixed versus separately. Thus, the objectives of this study were to evaluate the effects of level and form of CCDS supplementation on DMI, site of digestion, and ruminal fermentation in cannulated steers when fed low quality forages.

Materials and Methods

Animals and Diets. Five ruminally and duodenally cannulated Holstein steers were used in a 5 x 5 Latin square. Steers were offered ad libitum amount of a basal diet consisting of a mixture of 40% mature bluestem hay and 60% chopped mixed grass alfalfa hay (Table 1). Treatments consisted of a negative control (CON, no supplement), 0.2% BW CCDS mixed with the forage (DM basis), 0.4% BW CCDS mixed with the forage, 0.2% BW CCDS supplement fed separately in tanks, and 0.4% BW CCDS fed separately. Mixed rations were mixed at the ratio 1:0.55 (forage to CCDS) for the 0.2% mix diet and 1:1.1 for the 0.4% mix diet (as-fed basis).

Sampling and Collections. Individual ingredient samples were taken daily (approximately 200 g) and composited within period. Orts were taken daily, prior to morning feeding (0700), and sampled daily throughout 7-d collection period. Five days prior to and throughout collections, 8 g of chromic oxide was dosed ruminally twice daily at 0700 and 1900 via gelatin capsule (Torpac, Inc., Fairfield, NJ) for use as a digesta flow marker. Total fecal

collections were performed and total fecal output determined daily. Fecal sub-samples (10% of output; wet weight basis) were composited within steer during each period. Duodenal samples (200 mL) were collected over 4 d in a manner that allowed for every other hour in a 24-h period to be sampled.

Table 1. Analyzed nutrient content of forage and corn condensed distillers solubles (CCDS).

Item, %	Forage[1]	CCDS[2]
DM	87.7	33.6
	%, DM Basis	
Fat	ND[3]	15.6
Ash	6.5	6.9
CP	8.2	20.4
NDF	73.6	ND
ADF	47.6	ND
Ca	0.6	0.1
P	0.1	1.2
S	ND	1.2

[1]Forage consisted of 40% mature bluestem hay and 60% mixed grass alfalfa hay.
[2]CCDS = corn condensed distillers solubles
[3]ND = not determined.

Two hundred milliliters of Co-EDTA (1734 mg Co; Uden et al., 1980) were dosed intraruminally 2 h prior to feeding on d 6 of each collection period. Ruminal fluid samples (200 mL) were collected with a suction strainer at 0, 2, 4, 6, 8, 10, and 12 h post feeding, and pH immediately determined. Samples (200 mL) were acidified with 2 mL, 6.0 *N* HCl. A sub-sample (3 mL) of the initial, non-acidified ruminal fluid sample was collected and added to 0.75 mL metaphosphoric acid and frozen (-20˚ C) until VFA analysis.

On d 7 of each collection period, prior to morning feeding, ruminal evacuations were conducted to determine ruminal fill. Ruminal contents were removed, weighed, and sub-sampled. Sub-samples were obtained by hand mixing ruminal contents in 208-L containers and sampling from various locations. A grab sample was taken for DM, OM, ADF, and NDF analyses. A second ruminal content sample (4 kg) was taken and 2 L of formalin/ saline solution (3.7% formaldehyde/ 0.9% NaCl) was added (Zinn and Owens, 1986) for isolation of bacterial cells.

Laboratory Analysis. Diet, ort, and fecal samples were dried using a forced-air oven (55˚ C; The Grieve Corporation, Round Lake, IL) for 48 h. Dried samples were ground in a Wiley mill to pass through a 2-mm screen. Duodenal samples were lyophilized (Virtis Genesis 25LL; The Virtis Company, Inc., Gardiner, NY) and ground with a Wiley mill to pass through a 1-mm screen.

Diet, ort, duodenal, and fecal samples were analyzed for DM, ash, and N (Procedure numbers: 930.15, 942.05, 984.13, respectively; AOAC, 1997). Concentrations of NDF (Robertson and Van Soest, 1991, as modified by Ankom Technology, Fairport, NY) and ADF (Goering and Van Soest, 1970, as modified by Ankom Technology) were determined using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY) without sodium sulfite, with amylase, and without ash correction as sequentials. Chromic oxide concentrations were analyzed in duodenal samples by the spectrophotometric method (Fenton and Fenton, 1979).

Ruminal fluid samples were centrifuged at 20,000 x g for 20 min and supernatant taken for analysis of ammonia (Broderick and Kang, 1980). Ruminal VFA concentrations (Goetsch and Galyean, 1983) were quantified by gas chromatography (Hewlett Packard 5890A Series II GC, Wilmington, DE) using a capillary column. Cobalt was analyzed by methods described by Uden et al. (1980) with an air-plus-acetylene flame using atomic absorption spectroscopy (Model: 3030B; PerkinElmer, Inc., Wellesley, MA).

Ruminal content samples from total evacuations were analyzed for DM and ash (AOAC, 1997). Isolated bacterial cells and duodenal contents were analyzed for purines (Zinn and Owens, 1986) as a microbial marker.

Statistical Analysis. Data were analyzed as a 5 x 5 Latin square using the MIXED procedures of SAS (SAS Inst., Cary, NC). The model included diet and period as fixed effects and steer as the random effect. Data over time was analyzed as a repeated measures design using the MIXED procedures of SAS (SAS Inst., Cary, NC). The model included period, animal, diet, time, diet x time, and animal x period x diet with the random variable being animal. Orthogonal contrasts included control vs. supplemented treatments, mixed diets vs. CCDS fed seperately, 0.4% level vs. 0.2% level, and the interaction of method of feeding x level of CCDS.

Results and Discussion

Forage DMI (kg/d) tended ($P = 0.08$) to have an interaction of level of CCDS by method of feeding. Forage DMI (Table 2) was not affected ($P > 0.13$) by treatments, which is similar to studies by Freeman et al. (1992) and Köster et al. (1997) who reported no effects of RDP supplementation on forage DMI. By design, CCDS DMI was increased ($P < 0.01$) in both control versus supplemented steers and high versus low treatments. However, there was also an increase ($P < 0.01$) in CCDS DMI in steers fed CCDS separately compared to those fed mixed diets. Total DMI was increased ($P = 0.04$) in supplemented steers compared to control steers. Thus, total DMI was greater ($P = 0.04$) in steers fed CCDS separately than those steers fed mixed diets. No treatment effects for ruminal DM fill were observed ($P = 0.32$).

Fluid dilution rate (FDR; 10.8 ± 1.2%/h) was not affected ($P = 0.17$) by CCDS supplementation (Table 2). Gilbery et al. (2006) reported no effects of increasing CCDS levels on FDR, and other researchers (Köster et al., 1997; Bandyk et al., 2001) also reported no increase in FDR.

Apparent ruminal, true ruminal, and intestinal digestion of OM were not affected ($P > 0.22$) by treatments (Table 4). Total tract digestion was increased ($P = 0.01$) in steers fed CCDS separately compared to steers fed mixed diets. Gilbery et al. (2006) reported no effects of increasing levels of CCDS on OM digestion when CCDS was fed separately.

Apparent ruminal, true ruminal and total tract digestion of CP was increased ($P < 0.01$) in supplemented steers compared to control fed steers; whereas intestinal CP digestion was decreased in supplemented steers. Steers fed mixed diets had increased apparent ruminal ($P = 0.01$), true ruminal ($P = 0.05$), and total tract ($P < 0.01$) CP digestion compared to steers fed CCDS separately. However, steers fed mixed diets had decreased ($P = 0.04$) intestinal CP digestion. Microbial efficiency (14.8 ± 5.7 g of microbial N/kg OM truly fermented) was not affected by treatments. Total tract digestion of NDF and ADF was decreased (Table 4, $P < 0.03$) with CCDS supplementation.

Concentrations of NH_4 (3.89±0.72 mM) and total VFA (71.3±6.2 mM) were not affected ($P > 0.17$) by treatment (Table 3). Ruminal pH (6.74±0.09) was decreased ($P = 0.04$) in steers fed mixed diets compared to steers fed separately; whereas ruminal pH was not different between the control treatment and supplemented treatments ($P = 0.12$). Molar proportion of acetate was increased ($P = 0.02$) in supplemented steers compared to control fed steer; whereas molar proportion of butyrate increased in supplemented steers ($P < 0.01$) and those fed mixed diets ($P = 0.01$). Molar proportion of propionate tended to be greater in supplemented steers ($P = 0.06$), steers fed CCDS separately ($P = 0.07$), and steers fed high level of CCDS ($P = 0.05$). However, the acetate to propionate ratio was decreased ($P < 0.01$) in supplemented steers and increased ($P < 0.01$) in steers fed mixed diets. There was a time x treatment interaction for propionate ($P < 0.01$), butyrate ($P < 0.01$), and acetate to propionate ratio ($P = 0.01$). This was due to a magnitude response and was not thought to be biologically significant.

Results of this study suggest that use of CCDS for protein and energy supplementation increases intake and CP digestion but has minimal effects on fiber utilization and microbial efficiency in steers fed forage based diets.

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Table 2. Effects of corn condensed distillers solubles (CCDS) supplementation on DMI, ruminal fill, and fluid dilution rate in steers consuming a forage-based diet.

	Treatment[1]							Contrast[2]			
Item	CON	0.2% MIX	0.4% MIX	0.2% SEP	0.4% SEP	SEM[3]	*P*-value[4]	CON vs. SUP	MIX vs. SEP	HIGH vs. LOW	METH x LEV
Forage DM intake											
kg/d	6.75	5.99	6.43	6.28	5.03	0.66	0.130	0.123	0.381	0.232	0.081
% of BW	0.80	0.77	0.95	0.70	0.57	0.14	0.349	0.689	0.847	0.104	0.242
CCDS DM intake kg/d	0.00	0.93	2.38	1.61	3.14	0.20	<0.001	<0.001	<0.001	0.003	0.828
Total intake											
kg/d	6.75	6.92	8.81	7.89	8.17	0.76	0.043	0.041	0.038	0.736	0.111
% of BW	0.81	0.89	1.31	0.93	0.98	0.20	0.422	0.312	0.238	0.469	0.347
Ruminal DM fill, % of BW	16.36	11.25	11.96	9.29	9.98	2.73	0.315	0.052	0.778	0.431	0.997
Fluid dilution rate, %/h	9.17	12.04	12.01	9.51	11.42	1.18	0.173	0.089	0.372	0.148	0.361

[1]CON = forage only, 0.2% MIX = forage mixed with 0.2% BW CCDS supplement, 0.4% MIX = forage mixed with 0.4% BW CCDS supplement, 0.2% SEP = forage with 0.2% BW CCDS supplement fed separately, 0.4% MIX = forage with 0.4% BW CCDS supplement fed separately.
[2]CON vs. SUP = control treatment vs. all supplemented treatments, MIX vs. SEP = forage and CCDS mixed vs. forage and CCDS fed separately, HIGH vs. LOW = 0.4% BW CCDS level vs. 0.2% BW CCDS level, METH vs. LEV = method of feeding (mixed and fed separately) and CCDS supplementation level interaction.
[3]n = 5 observations.
[4]Probability value for the *F*-test of overall treatment.

Table 3. Effects of corn condensed distillers solubles (CCDS) supplementation on ruminal pH, NH_4 concentration, and VFA concentration in steers consuming a forage-based diet.

	Treatment[1]						*P*-value[4]			Contrast[2]			
Item	CON	0.2% MIX	0.4% MIX	0.2% SEP	0.4% SEP	SEM[3]	Trt	Time	Trt x Time	CON vs. SUP	MIX vs. SEP	HIGH vs. LOW	METH LEV
pH	6.80	6.77	6.64	6.77	6.72	0.09	0.092	0.010	0.168	0.120	0.042	0.407	0.34
NH_4, mM	3.08	4.66	4.20	3.27	4.26	0.72	0.173	<0.001	0.019	0.093	0.207	0.611	0.17
VFA													
Total, mM	73.23	70.40	75.50	69.66	67.69	6.18	0.231	0.017	0.047	0.388	0.529	0.102	0.16
Acetate	57.70	53.68	51.64	53.71	49.68	6.63	0.078	<0.001	0.947	0.016	0.117	0.602	0.59
Propionate	12.65	12.95	14.04	14.19	15.90	1.93	0.048	<0.001	<0.001	0.062	0.070	0.048	0.66
Butyrate	4.12	6.89	8.96	6.95	8.62	0.97	<0.001	<0.001	0.002	<0.001	0.007	0.819	0.73
Acetate: Proprionate[5]	4.59	4.14	3.77	3.83	3.31	0.12	<0.001	<0.001	0.006	<0.001	0.001	0.003	0.49

[1]CON = forage only, 0.2% MIX = forage mixed with 0.2% BW CCDS supplement, 0.4% MIX = forage mixed with 0.4% BW CCDS supplement, 0.2% SEP = forage with 0.2% BW CCDS supplement fed separately, 0.4% MIX = forage with 0.4% BW CCDS supplement fed separately.
[2]CON vs. SUP = control treatment vs. all supplemented treatments, MIX vs. SEP = forage and CCDS mixed vs. forage and CCDS fed separately, HIGH vs. LOW = 0.4% BW CCDS level vs. 0.2% BW CCDS level, METH vs. LEV = method of feeding (mixed and fed separately) and CCDS supplementation level interaction.
[3]n = 5 observations.
[4]Probability value for the *F*-test of overall treatment.
[5]Ratio of Acetate to Propionate.

Table 4. Effects of corn condensed distillers solubles (CCDS) supplementation on OM, CP, NDF, and ADF digestion in steers consuming a forage-based diet.

	Treatment[1]						Contrast[2]			
Item	CON	0.2% MIX	0.4% MIX	0.2% SEP	0.4% SEP	SEM[3]	*P*-value[4]	CON vs. SUP	MIX vs. SEP	HIGH vs. LOW	METH x LEV
OMI, kg/d	6.31	6.43	8.15	7.36	7.63	0.71	0.046	0.044	0.040	0.643	0.117
Digestion, % of intake											
Apparent ruminal	40.63	39.15	45.14	40.64	49.88	8.00	0.259	0.499	0.068	0.420	0.676
True ruminal	54.00	54.53	60.83	55.85	63.06	6.30	0.223	0.250	0.059	0.586	0.890
Intestinal	10.92	12.80	13.13	13.62	14.37	3.04	0.773	0.277	0.786	0.597	0.913
Total tract	49.88	51.95	57.29	50.50	64.25	5.93	0.036	0.110	0.011	0.402	0.209
CP digestion, % intake											
Apparent ruminal	-54.05	-15.97	-0.25	-27.84	3.46	1.74	0.003	<0.001	0.014	0.615	0.340
True ruminal	26.04	43.89	50.54	37.35	50.45	9.02	0.019	0.004	0.052	0.476	0.486
Intestinal	99.75	73.78	67.82	83.45	63.52	12.70	0.004	0.001	0.036	0.635	0.225
Total tract	43.82	57.81	66.71	51.80	66.98	5.65	<0.001	<0.001	0.002	0.3637	0.323
Microbial efficiency[5]	13.93	20.10	13.92	15.07	11.19	5.67	0.550	0.809	0.227	0.344	0.774
Digestion, % intake											
Total tract NDF	51.09	37.72	27.66	39.57	44.15	7.37	0.011	0.006	0.479	0.031	0.075
Total tract ADF	48.63	36.56	27.08	39.30	45.51	7.42	0.022	0.026	0.694	0.023	0.078

[1]CON = forage only, 0.2% MIX = forage mixed with 0.2% BW CCDS supplement, 0.4% MIX = forage mixed with 0.4% BW CCDS supplement, 0.2% SEP = forage with 0.2% BW CCDS supplement fed separately, 0.4% MIX = forage with 0.4% BW CCDS supplement fed separately.

[2]CON vs. SUP = control treatment vs. all supplemented treatments, MIX vs. SEP = forage and CCDS mixed vs. forage and CCDS fed separately, HIGH vs. LOW = 0.4% BW CCDS level vs. 0.2% BW CCDS level, METH vs. LEV = method of feeding (mixed and fed separately) and CCDS supplementation level interaction.

[3]n = 5 observations.

[4]Probability value for the *F*-test of overall treatment.

[5]Grams of microbial N per kilogram of OM truly fermented.

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IN SITU DISAPPEARANCE OF HIGH AND LOW AMYLOSE PEA VARIETIES AND THEIR EFFECTS ON FORAGE DIGESTIBILITY *IN VITRO*

A. V. Grove, A. L. Heryford, M. E. Dawe, and J.G.P. Bowman
Montana State University, Bozeman 59717

ABSTRACT: Two experiments evaluated, 1) the in situ DM and starch disappearance of high and low amylose pea varieties, and 2) the effect of high and low amylose pea varieties on DM and NDF digestibility, and pH in vitro. In Experiment 1, 2 ruminally-cannulated cows were used to evaluate DM and starch in situ digestibility of 4 pea varieties: Bolero (high amylose), MSU 89C (high amylose), MSU PBL27 (high amylose), and Majoret (low amylose). Pea samples were incubated in duplicate in the rumen of each of 2 cannulated cows for 0, 3, 6, 9, 24, and 48 h. Nonlinear regression was used to determine the immediately soluble fraction *A*, the potentially degradable fraction *B*, the undegraded fraction *C*, and the disappearance rate. There was no effect ($P > 0.12$) of pea variety on in situ DM or starch disappearance rate, lag time, or fraction *C*. Dry matter and starch fraction *A* were lowest ($P < 0.03$) for Majoret, intermediate for MSU 89C and MSU PBL27, and highest for Bolero, while DM and starch fraction *B* were highest ($P < 0.01$) for Majoret, intermediate for MSU 89C and MSU PBL27, and lowest for Bolero. In Experiment 2, 3 forages (alfalfa/grass hay, winter wheat, and wheat straw) were incubated in vitro with 3 pea varieties (Bolero, Majoret, MSU PBL27) at 4 pea:forage (0:100, 25:75, 50:50, and 75:25) for 24 and 48 h. The pH of the incubation fluid was lower ($P = 0.08$) when MSU PBL27 peas were included in the incubation vessels compared to inclusion of Majoret (6.80 vs 7.24). In vitro DM and NDF digestibility of forage were lower ($P < 0.001$) when incubated with MSU PBL27 than when incubated with Bolero or Majoret. In vitro DM and NDF digestibility and pH did not differ ($P > 0.87$) between pea:forage in the vessels at 24 or 48 h. Digestibility of peas did appear to be related to amylose content, and pea variety may affect ruminal pH and subsequent forage digestion.

Key Words: amylose, beef cattle, digestibility, field pea

INTRODUCTION

Montana ranks 2nd in the nation for dry pea production (NASS, 2008). Most of these peas enter the human food market; however, those that do not are often fed as a source of energy and protein to beef cattle, and can provide an economical alternative to high priced corn and barley. Pea starch is less digestible than barley starch (Ljøkel et al., 2003), and this reduction in digestibility could be due to differences in starch type: 77% amylopectin in barley and 65% amylopectin in peas (Ljøkel et al., 2003). Amylopectin is a branched glucan molecule of starch and is considered to be more digestible than its linear counterpart, amylose (Rooney and Pflugfelder, 1986).

Researchers at MSU are currently developing high amylose (low amylopectin) varieties of peas in hopes of reducing the negative effects of starch on forage digestion and helping to alleviate reduced pH in the rumen. Limited data is available on the ruminal digestibility of these newly developed pea varieties. The objectives of this study were to 1) determine DM, and starch *in situ* disappearance of low and high amylose pea varieties, and 2) determine the effects on low and high amylase pea varieties on forage digestion.

MATERIALS AND METHODS

Experiment 1

Two ruminally-cannulated cows were used to evaluate *in situ* digestibility of 4 pea varieties: Bolero (high amylose), MSU 89C (high amylose experimental line), MSU PBL27 (high amylose experimental line), and Majoret (low amylose). Samples from each variety were cracked using a Bühler mill (Bühler AG, Uzwil, Switzerland). Samples were analyzed for DM (AOAC, 1999), starch (Megazyme, Sidney, Australia), N (Leco Corporation, St. Joseph, MI), NDF, ADF (Van Soest et al., 1991), and particle size (Table 1).

Polyester bags (50 µm-pore-size; Ankom Technology, Macedon, NY) were labeled, dried in a 60° C forced-air oven for 24 h, individually weighed, and filled with 5 g of cracked pea sample. Pea samples were incubated in duplicate in the rumen of each of 2 cows for 0, 3, 6, 9, 24, and 48 h. In addition, a blank bag was included in each cow for each time point. Samples for 0 h were not placed in the rumen, but were simply rinsed in warm water.

After incubation, bags were washed in cold running water until the rinse water ran clear, squeezed gently by hand to remove excess water, dried for 48 h in a 60° C forced-air oven, and weighed to determine residue. Original samples and *in situ* residues were ground through a Wiley mill (1-mm and 0.5-mm screens) and analyzed for DM (AOAC, 1999), and starch (Megazyme, Sidney, Australia).

The DM and starch disappearance were fit to a model using PROC NLIN (SAS Inst. Inc., Cary, NC). Initial estimates of the rate constant (Kd) and lag time of DM and starch disappearance were necessary for model fitting and were calculated as described by Bowman and Firkins (1993). The basic model used was from Mertens and Loften (1980). Dry matter and starch were partitioned into 3 fractions, defined as immediately soluble (fraction *A*), disappearing at a measurable rate (fraction *B*), and undegradable (fraction *C*). The rate constant for fraction *B*,

lag time, and fractions *B* and *C* were determined from the nonlinear model, whereas fraction *A* was calculated as: 100 − fraction *B* − fraction *C*. Fractions *A*, *B*, and *C*, the rate constant for fraction *B*, and lag time were analyzed using PROC GLM of SAS, with treatment (pea variety) in the model. Means were separated using the least significant difference test when the treatment effect was $P \leq 0.05$.

Experiment 2

This experiment was designed to test the effects of pea variety and amount of peas added to the *in vitro* vessel on pH and subsequent IVDMD of 3 forages (wheat straw, alfalfa/grass hay, and winter wheat hay; Table 2). Forage samples were ground through a 1-mm screen and incubated in duplicate in an Ankom batch incubator (Ankom Technology, Macedon, NY) for 24 and 48 h. Cracked peas (Majoret, Bolero, or MSU PBL27) were added to the incubation vessels at levels to achieve pea:forage of 0:100, 25:75, 50:50, and 75:25. Two runs per pea variety were conducted. A pH reading was taken when forage samples were removed at 24 and 48 h. Forage samples were dried for 24 h in a 60° C forced-air oven, then weighed, and analyzed for DM (AOAC, 1999) and NDF (Van Soest et al., 1991). Data were analyzed using the GLM procedure of SAS with forage, pea variety, amount of peas, and all interactions included in the model. Means were separated using LSD when $P < 0.10$.

RESULTS AND DISCUSSION

Experiment 1

The chemical composition of pea varieties is presented in Table 1. Although not analyzed statistically, Bolero had lower starch content than the other 3 pea varieties, while the 2 new experimental lines had a lower N content and larger particle size compared to Bolero and Majoret.

The immediately soluble fraction of DM and starch were greatest ($P \leq 0.03$) for Bolero, intermediate for MSU 89C and MSU PBL27, and least for Majoret (Table 3). Dry matter and starch fraction *B*, the portion that disappeared at a measureable rate, was greatest ($P \leq 0.01$) for Majoret, intermediate for MSU 89C and MSU PBL27, and least for Bolero. No differences ($P \geq 0.12$) were found between the pea varieties in the undegradable DM or starch fraction, the rate of DM or starch disappearance, or lag time.

The higher soluble starch fraction of Bolero compared with Majoret conflicts with the proposed hypothesis that high amylose content would reduce rate of ruminal starch digestion. In agreement with our research, Phillipeau et al. (1998) reported that ruminal starch degradability was not related to amylose content of starch, but was influenced by other factors such endosperm texture, protein distribution in the endosperm, and particle size distribution.

Experiment 2

There were no interactions ($P \geq 0.32$) between pea variety, pea:forage, or type of forage for *in vitro* DM and NDF digestibility and pH measured after 24 or 48 h of incubation. *In vitro* DM digestibility was lowest ($P < 0.001$) for straw, intermediate for winter wheat, and highest for alfalfa/grass hay at 24 and 48 h of incubation (Table 4). *In vitro* NDF digestibility was lowest ($P < 0.001$) for straw, intermediate for alfalfa, and highest for winter wheat after 24 h of incubation and lower ($P = 0.02$) for straw than for alfalfa/grass hay and winter wheat at 48 h.

The pH was lower ($P \leq 0.07$) after 24 and 48 h of incubation when MSU PBL27 peas were included in the incubation vessels compared to inclusion of Majoret or Bolero (6.82 vs. mean 7.20, and 6.40 vs. mean 7.13, for 24 and 48 h, respectively; Table 5). *In vitro* DM and NDF digestibility of forages were lower ($P < 0.001$) when forages were incubated with MSU PBL27 than when incubated with Bolero or Majoret at both time points. *In vitro* DM and NDF digestibility and pH did not differ ($P > 0.87$) between pea:forage in the incubation vessels at 24 and 48 h. The pH remained at acceptable levels for cellulose digestion throughout the experiment (pH > 6.0; Owens and Goetsch, 1988); however, *in vitro* DM and NDF digestibility appeared to correspond to pH in the incubation vessels.

We hypothesized that Majoret would have greater starch digestibility due to a lower amylose content which could decrease pH and have negative effects on forage digestion; however, this was not the case. Majoret, a low amylose pea variety, had a lower immediately soluble starch fraction and a numerically higher pH *in vitro* compared to Bolero, a high amylose pea variety.

Our results do not support the hypothesis that high amylose content in pea varieties reduces starch digestion; however, there did appear to be differences in starch digestion and pH between pea varieties.

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Table 1. Chemical composition (DM basis) of pea varieties used in Experiments 1 and 2

Item	Bolero	MSU 89C	MSU PBL27	Majoret
DM, %	93.0	92.8	93.2	93.0
Starch, %	27.8	33.5	35.5	38.6
N, %	4.8	3.7	3.4	5.1
NDF, %	13.1	14.1	16.3	15.6
ADF, %	8.3	7.3	8.5	5.8
Particle size, um	1,184	1,429	1,309	1,212
Appearance	Wrinkled	Wrinkled	Wrinkled	Smooth

Table 2. Chemical composition (DM basis) of forages used in Experiment 2

Item	Alfalfa/grass hay	Winter wheat hay	Straw
DM, %	89.5	92.0	91.8
N, %	1.33	1.43	0.87
NDF, %	58.9	69.6	80.9
ADF, %	35.2	42.7	50.9
Lignin, %	4.2	5.1	5.5

Table 3. *In situ* disappearance of DM and starch in 4 pea varieties (Experiment 1)

	Variety					
	Bolero	MSU 89C	MSU PBL27	Majoret	SE	*P*-value
DM disappearance[1]						
Fraction *A*, %	23.2^{c}	19.1^{b}	16.5^{b}	9.5^{a}	1.99	0.03
Fraction *B*, %	76.5^{a}	81.0^{b}	83.4^{b}	89.0^{c}	1.35	0.01
Fraction *C*, %	0.3	0	0	1.6	0.80	0.53
K_d, h^{-1}	0.13	0.10	0.11	0.15	0.035	0.81
Lag time, h	0	0	0.2	0.2	0.11	0.61
Starch disappearance						
Fraction *A*, %	33.0^{c}	8.8^{b}	13.8^{b}	0.3^{a}	2.51	0.003
Fraction *B*, %	67.0^{a}	88.2^{b}	84.3^{b}	95.0^{c}	2.02	0.002
Fraction *C*, %	0	2.9	2.0	4.8	1.03	0.12
K_d, h^{-1}	0.11	0.13	0.10	0.15	0.034	0.77
Lag time, h	0	0.4	0.1	0.4	0.19	0.40

[1]Fraction abbreviations: *A* = immediately soluble fraction, *B* = fraction disappearing at a measurable rate, *C* = undegraded fraction, and K_d = disappearance rate of fraction *B*.

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

Table 4. *In vitro* DM and NDF digestibility of 3 forages after 24 and 48 h of incubation (Experiment 2)

	Forage				
	Straw	Alfalfa	Winter wheat	SE	*P*-value
DM disappearance, %					
24 h	15.6[a]	37.6[c]	32.5[b]	1.16	<0.001
48 h	25.6[a]	48.0[c]	41.9[b]	1.78	<0.001
NDF disappearance, %					
24 h	8.8[a]	15.1[b]	19.2[c]	1.48	<0.001
48 h	21.3[a]	29.9[b]	30.4[b]	2.48	0.02

[abc] Within a row, means without a common superscript letter differ ($P < 0.10$)

Table 5. Dry matter and NDF disappearance of forage, and pH of ruminal fluid inoculated with 4 levels of peas after 24 and 48 h of *in vitro* incubation (Experiment 2)

	Pea variety			Pea:forage					*P*-value	
	Bolero	PBL27	Majoret	0:100	25:75	50:50	75:25	SE	Pea variety	P:F
pH										
24 h	7.13[b]	6.82[a]	7.26[b]	7.17	7.10	7.05	6.95	0.125	0.07	0.76
48 h	7.03[b]	6.40[a]	7.23[b]	6.89	6.93	6.89	6.82	0.081	0.001	0.87
DMD,%										
24 h	30.1[b]	23.4[a]	32.2[b]	28.7	29.0	27.8	28.7	1.16	<0.001	0.93
48 h	40.8[b]	30.2[a]	44.6[b]	37.8	39.3	38.5	38.5	1.78	<0.001	0.96
NDFD,%										
24 h	16.3[b]	8.8[a]	18.1[b]	14.9	14.6	13.7	14.2	1.48	<0.001	0.96
48 h	30.0[b]	17.9[a]	33.7[b]	27.7	28.6	25.7	26.8	2.48	<0.001	0.90

[abc] Within a row and effect, means without a common superscript letter differ ($P < 0.10$)

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Digestibility of dormant warm season vegetation with addition of supplemental protein

P. H. Sanchez*, S. L. Lodge-Ivey, S. H. Cox, R. L. Dunlap II, C. A. Löest, and M. K. Petersen

Department of Animal and Range Sciences, New Mexico State University, Las Cruces

ABSTRACT: Six crossbred, mature beef cows fitted with rumen cannulas were used in a completely randomized design to evaluate the effect of a hand-fed cottonseed meal (CSM) based versus a self-fed mineral-fishmeal, on in situ OM and NDF disappearance and VFA production. Cows were randomly assigned to 1 of 2 pastures (n = 3), allowed to graze dormant native vegetation and were initially individually supplemented with 1) control (CON; 36% CP, 35% undegradable intake protein (UIP), 57% CSM, 21% wheat midds, 10% soybean meal, 9% molasses and 1.2% urea; DM basis) fed at 454 g/hd per d delivered three times a wk or 2) small supplement (SSP; 33% CP, 60% UIP, 50% fishmeal, 33% minerals, and 17% salt; DM basis) was self-fed and formulated to achieve a targeted daily intake of 115 g/hd. Ruminal extrusa was collected via total rumen evacuation from two ruminally cannulated cows, that were allowed to graze for 1 h, 2 weeks prior to implementation of in situ protocol. Nutrient analysis of extrusa showed 4.6% CP, 56.1% NDF and 14.9% ash; OM basis. Extrusa samples were placed in nylon in situ bags and incubated for approximately 0, 24, 40, 48, 64, 72 and 96 h. Rumen fluid samples were collected for VFA analysis at 0, 24, 48, 72 and 96 h during the in situ incubation. The rate of in situ OM disappearance was faster (P = 0.04) for CON than SSP (8.1 vs. 6.0 $\pm$ 0.007 %/h, respectively). In situ NDF disappearance did not differ by treatment (P = 0.36; 5.6 vs. 5.1 $\pm$ 0.004 %/h, respectively). Total VFA, acetate, propionate, and acetate to propionate ratio did not differ between treatments (P > 0.17). This study demonstrated the impact of a small package supplement, high in UIP, is comparable to 36% hand-fed supplement on forage NDF digestibility and VFA production.

Key Words: beef cattle, in situ, protein

Introduction

Cattle grazing western rangelands during the dormant growth season consume low quality forages creating a need for supplementation. Supplementation of protein can enhance the digestibility of dormant range forages (Owens et al., 1991; Waterman et al., 2006). Protein sources used in supplements possess characteristics influencing ruminal degradability and total tract digestibility. These distinctions are categorized as either degradable intake protein (**DIP**) or undegradable intake protein (**UIP**). In practice, DIP has been supplemented to ruminants grazing, low quality diets, resulting in improved forage digestibility (Swanson et al., 2004). Similar forage digestibility research has been conducted with UIP and results demonstrated that UIP often improves or is comparable to that of DIP supplementation (Schloesser et al., 1993; Alderton et al., 2000; Bohnert et al., 2002). High UIP supplements can enhance the efficiency of nitrogen utilization allowing gestating cows to maintain BW while grazing dormant winter range (Sawyer et al., 1998; Sawyer et al., 2005). Previous studies conducted at NMSU utilized a self-fed supplement consumed at 0.25 kg d^{-1} higher in UIP resulted in no change in cow production when compared to the traditional hand-fed supplement at 0.45 kg d^{-1} of a 36% CP range cube higher in DIP. The objective of this study was to evaluate effect of a hand-fed cottonseed meal (**CSM**) based supplement compared to a self-fed mineral-fishmeal supplement on ruminal in situ OM (OMD) and NDF (NDFD) disappearnce and VFA production of cows grazing dormant native range.

Materials and Methods

This experiment was conducted at the Corona Range and Livestock Research Center, Corona, NM with an average elevation of 2000 m and an annual precipitation of 400 mm. The rangeland is characterized as a piñon-juniper woodlands with the predominate vegetation being blue grama (Bouteloua gracilis) and wolftail (Lycurus phleoides). Sideoats grama (Bouteloua curtipendula), threeawn (Aristida spp.), and black grama (Bouteloua eriopoda) are considered to be the minor sources of vegetation according to Knox (1998).

Animal care and handling procedures were in accordance to guidelines set forth by New Mexico State University Intuitional Animal Care and Use Committee. Six crossbreed mature beef cows fitted with rumen cannulae were used in a completely randomized design. Cows were randomly assigned to 1 of 2 pastures (n = 3), allowed to graze dormant native range during January, 2009. Cows were supplemented individually with control (**CON**) or self-fed small supplement (**SSP**; Table 1). Cows supplemented with CON were fed 454 g/hd per d equivalent to 136.2 g of UIP delivered 3x/wk. The CON was a 36% CP range cube (35% UIP; 57% CSM, 21% wheat midds, 10% soybean meal, 9% molasses, 1.2% urea and fortified with trace vitamins and minerals) and were allowed ad libitum access to loose mineral. Cows supplemented with SSP were dosed with approximately 805 g directly into the rumen 7 days prior to sampling. The SSP was formulated to achieve a targeted daily intake of 115 g/hd equivalent to 69 g UIP (33% CP, 60% UIP, 50% fishmeal, 33% minerals and 17% salt).

Prior to the start of the in situ experiment 2 cows were held overnight in a pen with access to water but no feed. Cows were ruminally evacuated; digesta was placed in 133-L containers and was returned after sampling was completed. After evacuation cows were allowed to graze dormant native winter range for 60 min. Masticate samples were subsequently collected and used to estimate OMD and NDFD. Masticate samples were dried in a forced-air oven (50^0 C) to a constant weight, ground in a Wiley Mill (2-mm screen), and composited on an equal weight basis.

Five-gram samples were sealed in Dacron bags (10 x 20 cm, 50 $\pm$ 5 µm pour size; ANKOM, Fairport, NY). In situ bags were incubated within nylon washing bags (30.5 x 25.4 cm) for approximately 96, 88, 72, 64, 48, 40, 24 and 0 h. A 1 m piece of nylon cord was tied to the corner of the mesh bag with a rubber stopper tied to the opposite end, which was left outside the rumen to facilitate bag retrieval. All bags were removed at 0 h and washed (in the field) in cold water until water ran clear, 0 h bags were added to the mesh bag at washing. All bags were dried for 48 h in a forced-air oven (50^0 C) weighed and stored at room temperature for analysis of DM, OM and NDF of the residue. During the in situ experiment ruminal fluid was collected 0 (prior to the addition of the 96 h Dacron bag), 24, 48, 72 and 96 h to determine VFA concentration.

Laboratory analysis. Volatile fatty acid (VFA) concentration was determined by gas chromatography (Star 3400, Varian, Walnut Creek, CA) using the methods of May and Galyean, (1996). In situ masticate samples were analyzed for DM and OM (Methods 930.15 and 942.05; AOAC, 1997) Also, NDF analysis was preformed according to Van Soest et al. (1991) using an ANKOM 200 fiber analyzer (ANKOM Co.).

Calculations and Statistics. In situ OM and NDF disappearance (%/h) were estimated using the model of Mertens and Loften (1980). Statistical analyses were performed using the GLM procedure of SAS to determine the differences in mean responses in the hand-fed supplement verses self-fed supplement. Data analyzed were rate of OMD and NDFD, and VFA concentrations.

RESULTS AND DISCUSSION

Effects of protein supplementation on OMD and NDFD are shown in Table 2. The rate of OMD of CON fed cows was greater than SSP (8.1 vs. 6.0 $\pm$ 0.007 %/h, respectively, P = 0.04). However, treatment did not influence NDFD (P = 0.36; 5.6 vs. 5.1 $\pm$ 0.004 %/h, respectively). Caton et al. (1988) observed NDFD values of 3.4 %/h for cottonseed meal (45.5% CP) supplemented steers which was lower than our values. Differences detected in OMD vs no difference in NDFD could be due to NDFD being a more sensitive measure of fiber digestion in the rumen. In situ OMD is also clouded by the disappearance of protein and other carbon substrates.

Total VFA concentrations and molar proportions of acetate, propionate and acetate: propionate ratios did not differ ($P \geq 17$) by treatment. The lack of effect due to type of protein supplement fed on VFA concentration is in agreement with other authors who reported comparable VFA concentrations (Bohnert et al., 2002; Caton et al., 1988). Affects of supplement type on acetate and propionate molar proportions contradict the findings of Köster et al. (1996) where DIP supplementation resulted in a decrease in acetate and an increase in propionate. However, no change in acetate or propionate molar proportions in DIP vs UIP supplemented cattle agrees with Bohnert et al., 2002.

IMPLICATIONS

Type of protein supplementation (i.e. UIP vs DIP), method of delivery and quantity of supplement did not seem to compromise rumen fermentation or NDFD. Although rate of OMD was greater with DIP supplementation, it appears that the cost of the supplement and delivery are important criteria for deciding the appropriate supplement to use since effects on ruminal function appear to be equitable.

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Table 1. Composition of New Mexico State University (NMSU) mineral, Hand-fed Cottonseed meal based range cube, and self-fed small supplement

Control 36% CP[1]	
Cottonseed meal, %	57.0
Wheat midds, %	21.0
Soybean meal, %	10.0
Molasses, %	9.0
Urea, %	1.2
Fortified trace - vitamins and minerals, %	1.8
Self-fed small supplement 35% CP[2]	
Fishmeal, %	50.0
NMSU Mineral, %	33.0
Salt, %	17.0
NMSU Mineral	
Calcium, maximum, %	11.5
Phosphorus, minimum, %	8.0
Magnesium, minimum, %	2.0
Copper, ppm	1,000.0
Zinc, ppm	1,000.0
Manganese, ppm	2,500.0
Selenium, ppm	13.0
Vitamin A, units/kg	54545.5

[1]UIP 35%
[2] UIP 60%

Table 2. Effects of 454 g/hd per d of hand-fed cottonseed meal based or 115 g/hd per d self-fed mineral-fishmeal on in situ OM disappearance, in situ NDF disappearance and VFA concentrations

	Treatment			
Measurement	CON	SSP	SE	*P*-value
ISOMD[1], %/h	8.1	6.0	0.007	0.04
ISNDFD[2], %/h	5.6	5.1	0.004	0.36
Total VFA, m*M*	66.8	82.2	10.90	0.15
Acetate, molar %	76.3	75.1	0.86	0.17
Propionate, molar %	15.9	16.1	0.74	0.77
Acetate:Propionate, mol/mol	4.8	4.7	0.21	0.47

[1]ISOMD = in situ organic matter disappearance
[2]ISNDFD = in situ neutral detergen fiber disappearance

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FEEDING CHILE PEPPER BYPRODUCT WITH ALFALFA HAY DECREASES TOTAL TRACT DIET DIGESTIBILITY IN BEEF STEERS

B. C. Graham, D. Cazac, C. P. Mathis, and C. A. Löest
New Mexico State University, Las Cruces, NM

ABSTRACT: Chile pepper byproduct is a potential feed source for cattle in the southwestern United States. The objective was to evaluate effects on increasing amounts of culled chile pepper pods (*Capsicum annuum*; CHILE) on rumen fermentation and total tract diet digestibility. Five ruminally cannulated Angus-cross steers (529 ± 10 kg initial BW) were housed individually in pens and were fed twice daily at 1.5% of BW/d (DM basis) one of the following combinations of alfalfa hay (62% NDF and 18.7% CP, DM basis) and CHILE (45% NDF and 16.7% CP, DM basis) on a DM basis: 1) 100:0, 2) 75:25, 3) 50:50, 4) 25:75, and 5) 0:100. The experiment was a 5 × 5 Latin square with 14-d periods, which allowed 9 d for adaption to dietary treatments and 5 d for collection of feed, orts, and feces. Samples of rumen fluid were collected at 0, 3, 6, 9, and 12 h after feeding on d 14 of each period. Rumen pH decreased when CHILE exceeded 50% of the diet (quadratic; $P = 0.02$). Concentrations of acetate, propionate, butyrate, and total VFA were lower when CHILE comprised 25 to 50% of the diet, then increased when CHILE was increased from 50 to 100% of the total diet (quadratic; $P < 0.01$). Although steers were limit-fed at 1.5% of BW/d, DM, OM, NDF, ADF, and N intakes decreased (linear; $P < 0.01$), fecal excretion of DM, OM, NDF, ADF, and N increased (linear; $P < 0.01$), and total tract apparent digestibility of DM, OM, NDF, ADF, and N decreased (linear; $P < 0.01$) when CHILE was added from 0 to 100% of the total diet. These results indicate that feeding chile pepper byproduct in combination with alfalfa hay negatively affects total tract diet digestibility in steers. Although chile pepper byproducts are relatively inexpensive, the value of this feed for cattle will also depend on the impact on performance responses.

Key Words: chile pepper byproduct, diet digestibility, steer

INTRODUCTION

Byproducts provide an opportunity to reduce feed costs relative to traditional feedstuff commodities for livestock while maintaining performance goals. Chile peppers (*Capsicum annuum*) are a potential byproduct feed for cattle in the southwestern United States. According to National Agricultural Statistics Service (2009), average production of chile peppers from 2006 through 2008 was 200,000 tons. As a southwest ethnic food product, much of the chile is processed (sorted, peeled, deseeded, and deveined) prior to consumption, which results in 15 to 25% waste (culled pods, peels, veins, leaves, seeds, and stalks).

Meija (1995) showed that feeding dairy cows an alfalfa-based diet with 20% chile waste tended to increase feed intake, and Cazac et al. (2004) demonstrated that addition of 20% chile pepper byproduct to cattle diets does not negatively impact feed preference. According to Hill and Löest (2003), the calculated energy value of chile pepper byproduct is similar to corn silage, and Cazac et al. (2005) demonstrated that ruminal degradation of chile pepper byproduct is greatest when cows are fed forage-based diets, and lowest when cows are consuming concentrate-based diets. With the exception of these studies, no research has evaluated digestibility of diets containing chile pepper byproducts. The objective of this experiment was to evaluate the effects of increasing amounts of chile pepper byproduct in a forage-based diet on total tract apparent digestibility.

MATERIALS AND METHODS

Animals, Facilities, and Treatments

Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Five ruminally-cannulated Angus-cross steers (529 ± 10 kg initial BW) were used in a 5 × 5 Latin square. Steers were randomly assigned to individual pens (15 × 35 m) with partial shade and automatic water troughs. Animals were limit-fed twice daily (1.5% of BW/d, DM basis) one of the following combinations (Table 1) of alfalfa hay and culled chile pepper pods (ratio on DM basis): 1) 100:0, 2) 75:25, 3) 50:50, 4) 25:75, and 5) 0:100. The chile pepper pods were culled by a processing plant (Biad Chile Ltd. Co., Mesilla Park, NM) due to inconsistency in size, color, stage of maturity or other factors such as damaged pods. Experimental periods were 14 d, which allowed 9 d for adaption to dietary treatments and 5 d for collection of feed, orts, and feces.

Collections

Steers were fitted with harnesses and collection bags for total collection of feces from d 10 through 14 of each period. A representative sample of daily fecal composite (5%) was frozen for later analysis. Also, samples of alfalfa hay, chile pepper pods, and feed refusals were collected and frozen daily during the 5-d collection period.

To determine ruminal fermentation characteristics, samples of rumen fluid were collected at 0, 3, 6, 9, and 12 h after feeding on d 14 of each period. Rumen fluid was strained through 4 layers of cheesecloth and the pH was measured (Hanna pH meter Model 9042 MP, Van Nuys, CA). Strained rumen fluid (10 mL) was mixed with 25% metaphosphoric acid (2 mL) and frozen until analysis.

Table 1. Dietary treatments

	Alfalfa:Chile				
Item, % of DM	100:0	75:25	50:50	25:75	0:100
Ingredient					
Alfalfa hay	100	75	50	25	0
Chile pods	0	25	50	75	100
Nutrient[1]					
OM	88.2	85.3	82.4	79.5	76.6
NDF	62.1	58.0	53.8	49.7	45.5
ADF	39.5	38.5	37.6	36.6	35.6
CP[2]	18.7	18.2	17.7	17.2	16.7

[1]Weighted average based on the nutrient analysis (DM basis) of the alfalfa hay (88.2% OM, 62.1% NDF, 39.5% ADF, and 18.7% CP) and culled chile pepper pods (76.6% OM, 45.5% NDF, 35.6% ADF, and 16.7% CP).
[2]Nitrogen analysis × 6.25.

Sample Analysis

Before analysis, samples of diet, orts, and feces that were collected daily for 5 d were thawed and composited for each steer within period. Composite samples were dried at 55°C for 72 h in a forced-air oven (Model #POM-326F, Blue M Electric Company, Blue Island, IL), allowed to air-equilibrate, weighed to determine moisture loss, and then ground to pass a 2-mm screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ).

Ground diet, orts, and fecal samples were analyzed for DM (105°C for 24 h) in a convection oven (Precision Scientific, Chicago, IL), and OM (500°C for 8 h) in a muffle furnace (Thermolyne Corp., Dubuque, IA). Sample concentrations of NDF and ADF were determined using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY), and N concentrations were analyzed via total combustion (LECO FP528, LECO Corp., St. Joseph, MI).

Rumen fluid was analyzed for VFA concentration (May and Galyean, 1996) using gas chromatography (Varian 3400, Walnut Creeck, CA). Concentrations of NH_3 in rumen fluid were analyzed using a micro plate reader (ELX 808 Ultra Microplate Reader, Bio-Tek Instruments, Winooski, VT) as outlined by May and Galyean (1996).

Statistical Analysis

The experiment was a 5 × 5 Latin square, and all data was analyzed statistically using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Steer was the experimental unit.

For dietary intake, feces, and apparent digestibility, the statistical model included period and diet as fixed effects, and steer as the random effect. Orthogonal contrasts were used to determine linear, quadratic, and cubic effects of dietary treatments.

Rumen fermentation data was analyzed using repeated measures with first order autoregressive covariance structure. The statistical model included period, diet, h, and the diet × h interaction as fixed effects, and steer within period × diet as the random effect. When there were no diet × h interactions, orthogonal contrasts were used to determine linear, quadratic, and cubic effects of dietary treatments. Data are presented as least squares means, and differences were considered significant at $P < 0.05$.

RESULTS

No diet × h interactions ($P \geq 0.17$) occurred for measures of rumen fermentation. Rumen pH decreased (quadratic; $P = 0.02$) when the amount of chile pepper pods exceeded 50% of the total diet, but rumen NH_3 concentrations were not affected ($P \geq 0.13$) by dietary treatments (Table 2). Concentrations of acetate, propionate, butyrate, and total VFA were lower when chile pepper pods comprised 25 to 50% of the diet, then increased when the amount of chile pepper pods was increased from 50 to 100% of the total diet (quadratic; $P < 0.01$). Dietary intakes of DM, OM, NDF, ADF, and N decreased (linear; $P < 0.01$), fecal DM, OM, NDF, ADF, and N excretion increased (linear; $P < 0.01$), and total tract apparent digestibility of DM, OM, NDF, ADF, and N decreased (linear; $P < 0.01$) when chile pepper pods were increased from 0 to 100% of the total diet (Table 3).

DISCUSSION

Byproducts provide an opportunity to reduce feed costs relative to traditional feedstuff commodities. However, various factors such as palatability and variability in both nutrient composition and nutrient availability (e.g. digestibility) may negatively impact animal performance, therefore limiting its value as a feed for livestock (Löest and Mathis, 2003). In the current study, linear decreases in both nutrient intake and nutrient digestibility when chile pepper pods replaced alfalfa hay demonstrated that this byproduct may have such negative effects. Decreases in DM intake occurred regardless of our attempts to minimize feed intake differences among treatments by limiting the daily DM offered to 1.5% of the average BW of steers. Although the pungency of chile peppers may affect palatability and consequently feed intake, Meija (1995) showed that 20% chile waste in an alfalfa-based diet tended to increase feed intake of dairy cows, and Cazac et al. (2004) demonstrated that addition of 20% chile pepper pods to diets does not negatively impact feed preference of beef cows.

Decreases in DM intake when increasing amounts of chile pepper pods replaced alfalfa hay in the diet of steers may be due to the low DM content of chile pepper pods (± 35% DM), or altered total tract passage rates, or decreased total tract diet digestibility, or all of the above. The total tract apparent digestibility of DM, OM, NDF, ADF, and N in chile pepper pods was approximately 30, 41, 64, 70, and 28% lower than the digestibility of these nutrients in alfalfa hay. When increasing amounts of chile pepper pods replaced alfalfa hay, the decreases in fiber digestibility were most dramatic. According to Hoover (1986), growth of rumen fibrolytic microbes and digestion of fiber decreases when rumen pH falls below 6.0. In our study, rumen pH decreased below 6.0 when the amount of chile pepper pods was increased from 50 to 100% of the total diet. Therefore, an altered rumen environment may be partly responsible for decreased fiber digestibility.

Linear changes in total tract apparent digestibility of nutrients indicated that the overall digestibility of the combinations of alfalfa hay and chile pepper pods reflects the weighted average of the digestibility of each feed alone. The absence of quadratic or cubic effects suggests that the combinations of alfalfa hay and chile pepper pods used in this study had no positive or negative associative effects on digestibility. Therefore, it appears that chile pepper pods did not affect the digestibility of alfalfa hay, nor did alfalfa hay affect the digestibility of chile pepper pods.

In conclusion, feeding chile pepper byproduct (culled pods) in combination with alfalfa hay negatively affects total tract diet digestibility in steers. Although chile pepper byproducts are relatively inexpensive, the value of this feed for cattle also depends on its impact on performance responses.

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Table 2. Effects of increasing amounts of chile pepper byproduct on rumen fermentation characteristics of steers

	Alfalfa:Chile[1]						Contrasts[2]		
Item	100:0	75:25	50:50	25:75	0:100	SEM	Lin.	Quad.	Cub.
pH	6.07	6.06	6.06	5.88	5.73	0.051	<0.01	0.02	0.93
NH_3, m*M*	0.67	0.74	0.68	0.78	0.57	0.071	0.51	0.13	0.43
Total VFA, m*M*	53.01	44.74	44.78	50.48	63.25	3.34	0.01	<0.01	0.90
Acetate	39.84	33.46	32.88	36.87	45.50	2.48	0.05	<0.01	0.87
Propionate	7.74	6.32	6.79	7.77	9.54	0.573	0.01	<0.01	0.53
Butyrate	3.80	3.31	3.57	4.30	6.51	0.397	<0.01	<0.01	0.56

[1]Dietary DM proportions of alfalfa hay and culled chile pepper pods limit-fed twice daily to steer (see Table 1).
[2]Linear, quadratic, and cubic contrasts.

Table 3. Effects of increasing amounts of chile pepper byproduct on total tract diet digestibility in steers

	Alfalfa:Chile[1]						Contrasts[2]		
Item	100:0	75:25	50:50	25:75	0:100	SEM	Lin.	Quad.	Cub.
Intake, kg/d									
DM	7.87	7.80	7.61	7.32	7.05	0.13	<0.01	0.35	0.74
OM	6.94	6.66	6.27	5.82	5.39	0.12	<0.01	0.43	0.76
NDF	4.88	4.53	4.11	3.64	3.19	0.082	<0.01	0.45	0.76
ADF	3.10	3.00	2.86	2.68	2.50	0.048	<0.01	0.30	0.79
N	0.236	0.227	0.216	0.201	0.189	0.0038	<0.01	0.47	0.70
Feces, kg/d									
DM	3.03	3.30	3.57	3.91	4.01	0.16	<0.01	0.57	0.55
OM	2.46	2.70	2.94	3.20	3.32	0.11	<0.01	0.48	0.61
NDF	2.16	2.24	2.27	2.53	2.55	0.12	<0.01	0.78	0.57
ADF	1.55	1.67	1.84	2.08	2.13	0.095	<0.01	0.81	0.34
N	0.068	0.080	0.083	0.088	0.092	0.0045	<0.01	0.36	0.64
Apparent digestibility, %									
DM	61.4	57.7	53.1	46.4	43.2	2.2	<0.01	0.87	0.45
OM	64.6	59.4	53.2	44.6	38.4	1.7	<0.01	0.43	0.49
NDF	55.6	50.6	44.4	29.7	20.2	2.7	<0.01	0.05	0.38
ADF	49.8	44.1	35.2	21.8	14.8	3.2	<0.01	0.46	0.25
N	71.1	64.7	61.6	56.8	51.4	2.1	<0.01	0.96	0.57

[1]Dietary DM proportions of alfalfa hay and culled chile pepper pods limit-fed twice daily to steer (see Table 1).
[2]Linear, quadratic, and cubic contrasts.

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EFFECTS OF EARLY LACTATION ON DMI, DM DIGESTION, AND RUMINAL DYNAMICS OF PRIMIPAROUS BEEF HEIFERS FED LOW-QUALITY, WARM-SEASON GRASS HAY

D. R. Linden[1], K. C. Olson[1], J. R. Jaeger[2], L. W. Murray[3], D. E. Anderson[4], L. A. Pacheco[1], K. E. Holcomb[4], J. W. Bolte[2], N. A. Sproul[1], and M. D. Thomas[1],

[1] Dept. of Animal Sciences and Industry, Kansas State University, Manhattan, KS
[2] Agricultural Research Center, Hays, Kansas State University, Hays, KS
[3] Dept. of Statistics, Kansas State University, Manhattan, KS
[4] Dept. of Clinical Sciences, Kansas State University, Manhattan, KS

ABSTRACT: Angus X heifers (n = 11; initial BW = 504 ± 40 kg) fitted with ruminal cannulae were individually fed chopped, warm-season grass hay (6.5 % CP and 36.8% ADF) ad libitum for 68 d postpartum. Six heifers were lactating and 5 heifers were non-lactating. Total tract DM digestibility (DMD), ruminal VFA, ruminal NH_3, particulate passage, and fluid dilution rate were measured every 14 d. Lactating and non-lactating heifers increased (period main effect - $P < 0.01$) DMI over the course of the study; however, DMI was similar ($P = 0.39$) between treatments. Apparent total-tract DMD generally increased over time but the magnitude of the response was influenced by lactation status (treatment x period - $P < 0.01$). Likewise, ruminal NH_3 increased (period main effect - $P < 0.01$) during the study; however, lactating heifers had less (treatment main effect - $P = 0.03$) ruminal ammonia compared with non-lactating heifers. Total ruminal VFA concentration was similar (treatment main effect - $P = 0.97$) between treatments and generally increased throughout the study (treatment x period - $P < 0.01$). Ruminal acetate generally increased (period main effect - $P < 0.01$) over time but was similar (treatment main effect - $P = 0.21$) between treatments. Ruminal molar proportions of propionate and butyrate varied over time (period main effect - $P < 0.01$) but were not influenced (treatment main effect - $P > 0.12$) by lactation status. Conversely, non-lactating heifers had greater (treatment main effect - $P < 0.01$) ruminal molar proportions of minor VFA than lactating heifers. Particulate passage, mean particulate-retention time, and fluid dilution rates were similar ($P > 0.51$) for lactating and non-lactating heifers. These data were interpreted to suggest that the dramatic increase in DMI and resulting changes in ruminal fermentation that are characteristic of beef cows during early lactation may not occur in beef heifers.

Key Words: Heifers, Intake, Lactation, Passage Rate, Ruminal Fermentation

Introduction

Lactating cows require nearly 20% greater maintenance energy when compared to non-lactating cows (Montano-Bermudez et al., 1990; NRC, 2000). Moreover, increased milk production is usually associated with significantly increased intake by mature cows (Wagner et al., 1986; Hatfield, et al., 1989). Full understanding of changes in DMI, DMD, ruminal fermentation, and digesta passage rates in primiparous heifers is needed to optimize postpartum nutrition and maximize rebreeding in order to maintain beef heifers on a 12-mo calving interval. The objective of our study was to measure the effects of early lactation on DMI, DMD, ruminal fermentation, and passage rate by primiparous beef heifers.

Materials and Methods

Animals and Diet. All procedures used in the care, handling, and sampling of animals in our study were approved by the Kansas State University Institutional Animal Care and Use Committee. Commercial Angus heifers (n = 11; average initial BW 504 ± 40 kg) were individually fed chopped warm-season hay (approximate particle length = 10 cm; 6.5 % CP, 36.8% ADF) for an average of 68 d postpartum. Heifers were housed indoors in individual tie-stalls (approximately 2 x 1.2 m) in an environmentally-controlled barn throughout the study (average temperature 25°C; average humidity 72%). Heifers were fed in individual feed bunks. Hay was maintained in a covered barn before and after chopping. Orts were weighed daily immediately prior to feeding at 0700. Water and trace-mineralized salt were available *ad libitum*. Heifers were weighed every 2 wk throughout the study; body condition scores were also assigned at that time as the average score given by three trained observers using a 9-point scale (1 = emaciated and 9 = extremely obese; Neumann and Lusby, 1986). Treatment assignments were based on lactation status. Six heifers began the study immediately after parturition; these were inseminated by transcervical AI after being synchronized. Estrus was synchronized using the 7-11 Co-Synch protocol as reported by Eborn and Grieger (2007). In addition, 5 heifers were non-pregnant, non-lactating controls.

Calves were removed permanently from dams at 24 h of age. Lactating heifers were milked by machine twice daily thereafter to approximate the energy demand created by a nursing calf. Oxytocin injections were given 1-min prior to commencing milking to ensure milk let down.

The postpartum period was divided into 5 14-d data collection periods. Total fecal output was estimated on d 9-12 using acid detergent-insoluble ash (ADIA) as an internal marker. Fecal grab samples were manually collected from the rectum every 4 h. Fecal samples were dried for 72 h in a forced air oven at 55°C to determine DM.

Ruminal fermentation and fluid dilution rates were characterized on d 13 of each data collection period. Cobalt-EDTA was used as an external marker of the fluid phase of ruminal digesta (Uden et al. 1980); marker was pulse dosed at a rate of 6.5 g at 0800. Ruminal fluid samples were obtained from three areas of the ventral rumen immediately prior to marker dosing (0 h) and 4, 8, 12, 16, and 20 h after dosing. Fluid was strained through four layers of cheesecloth and separated into two aliquots: 10 mL of were frozen for Co analysis and 10 mL were combined with 2 mL of 25% (wt/vol) metaphosphoric acid for VFA and NH_3 analysis. Ruminal fluid aliquots were frozen immediately after collection.

Ruminoreticular fill and ruminal ADIA passage rates were measured on d 14 of each collection period. Fill was determined by complete manual evacuation of digesta (solid and liquid fractions) via the ruminal fistula immediately prior to and 4 h after the daily feeding. Contents were weighed and mixed; 4 subsamples were subsequently collected. After sampling, all digesta was replaced in the rumen. Ruminal content DM was determined by drying subsamples in a 55°C forced air oven for 72 h. Ruminal DM fill was estimated by multiplying ruminal digesta DM by the total weight of ruminal digesta. Ruminal fluid fill was estimated as the difference between total weight of ruminal digesta and ruminal DM fill. Ruminal particulate passage rate was determined by measuring the ratio of ADIA ingested to ADIA in ruminal digesta.

Laboratory Analyses. Forage, orts, fecal, and ruminal samples were dried for 72 h in a forced air oven at 55°C and ground to pass a 1-mm screen (Model 4 Wiley mill; Thomas Scientific, Swedesboro, NJ, USA). Crude protein was determined by combustion (AOCA, 1980). Neutral-detergent fiber and ADF were determined using an Ankom Fiber Analyzer (Ankom^{200}, Macedon, NY, USA).

Ruminal fluid samples were thawed at room temperature for 2 h and centrifuged at 39,000 x g for 20 min. Ammonia concentration in the supernatant was determined using an autoanalyzer (Seal Analytical, Mequon, WI, USA; Broderick and Kang, 1980). Ruminal VFA concentrations were determined by gas chromatography per Supelco column specifications (Sigma Aldritch, St. Louis, MO, USA; column temp = 130°C, injection and detector temp = 250°C; column = 6' x ¼", 4mm ID glass packed with GP 10%; carrier gas = helium).

Cobalt content of ruminal fluid was determined by atomic absorption (Perkin Elmer Atomic Absorption Spectrometer 3110, Waltham, MA, USA; Wavelength = 240.7 nm, linear range = 3.5 mg/L, slit size = 0.2 nm; air-acetylene flame). The natural logarithm of cobalt concentration was regressed against sampling time to calculate fluid dilution rates (Warner and Stacey, 1968).

Statistical Analyses. Data were analyzed as a 6-period, 2-treatment completely random design using the MIXED procedure of SAS (SAS 9.1, 2009). Class variables included treatment, animal, and period. The model statement included terms for treatment, period, and treatment x period. Intake and DMD data were summarized as the means for each 2-wk period relative to the time of actual calving.

Data describing ruminal fermentation and passage rates were analyzed as a split-plot arrangement of a completely random design using the MIXED procedure of SAS. Whole plot effects included animal, period, and treatment. Subplot effects were time and treatment x time. Whole plot effects were tested using animal x period x treatment. Residual error was used to test subplot effects. When significant F-tests ($P < 0.05$) were observed, the method of least significant difference was used to partition treatment sums of squares.

Results and Discussion

Dry Matter Intake and Digestion. Lactating and non-lactating heifers increased (period main effect - $P < 0.01$) DMI over the course of the study; however, DMI was similar ($P = 0.39$) between treatments (Figure 1). Marston and Lusby (1995) also reported that beef heifers increased DMI until 6 wk postpartum. Conversely, Vanzant et al. (1991) and Hunter and Siebert (1986) reported a 17% increase in DMI by heifers and a 25% increase in DMI by cows, respectively, during the postpartum period. The postpartum increase in DMI was explained by the increase in energy requirements associated with milk production (Vanzant et al., 1991; Johnson et al., 2003).

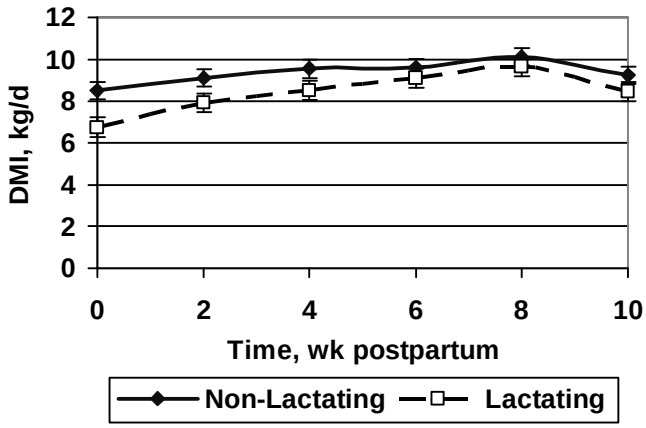

Figure 1. Dry matter intake by primiparous beef heifers from parturition to 10 wk postpartum.

Apparent total-tract DMD generally increased over time but the magnitude of the response was influenced by lactation status (treatment x period - $P < 0.01$; Figure 2). Diet digestibility was similar to that reported by Johnson et al. (2003) for primiparous beef heifers during early lactation. Vanzant et al. (1991) reported that OMD did not differ between lactating and non-lactating heifers 26 d post-partum. Colucci et al., (1982) reported that mature

dairy cows experienced a post-partum depression in DMD.

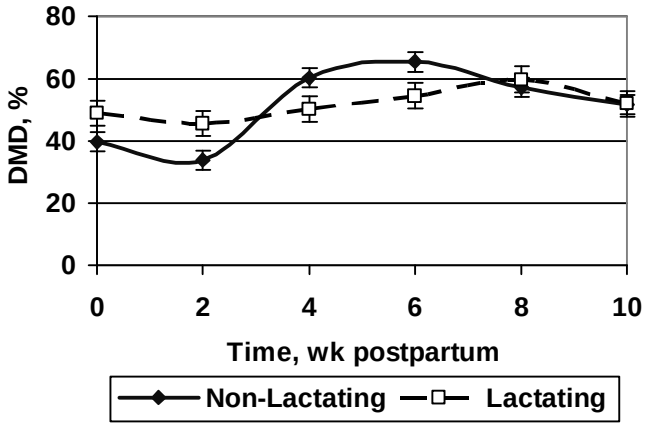

Figure 2. Dry matter digestibility by primiparous beef heifers from parturition to 10 wk postpartum.

Ruminal Fermentation. Ruminal NH_3 increased (period main effect - $P < 0.01$) over time during the study; however, lactating heifers had less (treatment main effect - $P = 0.03$; Table 1) ruminal ammonia than non-lactating heifers. Vanzant et al (1991) reported similar results. This decrease in ruminal NH_3 may be associated with an increase in ammonia flowing out of the rumen.

Total ruminal VFA concentration was similar (treatment main effect - $P = 0.97$) between treatments and generally increased throughout the study (treatment x period - $P < 0.01$; Table 1). Vanzant et al., (1991) reported that there were no differences in total ruminal VFA concentration between lactating and non-lactating heifers.

Ruminal acetate generally increased (period main effect - $P < 0.01$) over time but was similar (treatment main effect - $P = 0.21$) between treatments (Table 1). Vanzant et al. (1991) reported lesser proportions of acetate in lactating heifers compared with non-lactating heifers.

Ruminal molar proportions of propionate and butyrate varied over time (period main effect - $P < 0.01$) but were not influenced (treatment main effect - $P > 0.12$) by lactation status (Table 1). In contrast, Vanzant et al. (1991) reported greater proportions of propionate in lactating heifers. Decreased molar proportions of ruminal butyrate have been reported in lactating cows (Ingvartsen, 2006). The decrease was attributed to both increased ruminal absorption of butyrate and decreased DMI. Non-lactating heifers had greater (treatment main effect - $P < 0.01$) ruminal molar proportions of minor VFA than lactating heifers (Table 1).

Passage Rate. Particulate passages (Figure 3) and fluid dilution rates (Figure 4) were similar ($P > 0.51$) for lactating and non-lactating heifers. Vanzant et al. (1991) reported lactating heifers had greater particulate passage rate when compared with non-lactating heifers. This may have been driven by increased DMI (Okine and Mathison, 1991). Vanzant (1991) reported greater fluid dilution rates in lactating cows than in non-lactating cows. Roughly equal DMI ($P = 0.39$) between lactating and non-lactating heifers in our study was probably the reason for similarities in particulate passage and fluid dilution rate.

Implications

These data were interpreted to suggest that the dramatic increase in DMI and resulting changes in ruminal fermentation that are characteristic of beef cows during early lactation may not occur in beef heifers.

Table 1. Ruminal molar proportions of acetate, propionate, butyrate, and minor VFA* in primiparous beef heifers postpartum.

	Treatment Means				
Item	Lactating	Non-Lactating	SE	*P*	Effect
Ammonia, mM	0.63	1.00	0.11	0.03	Treatment
Total VFA, mM	73.78	73.67	2.47	0.97	Treatment
Acetate, % of total VFA	69.05	68.44	0.33	0.21	Treatment
Propionate, % of total VFA	35.67	35.65	0.15	0.92	Treatment
Butyrate, % of total VFA	11.24	11.54	0.13	0.12	Treatment
Minor VFA*, % of total VFA	2.28	2.85	0.03	<0.01	Treatment

*Minor VFA included isobutyrate, valerate, and isovalerate

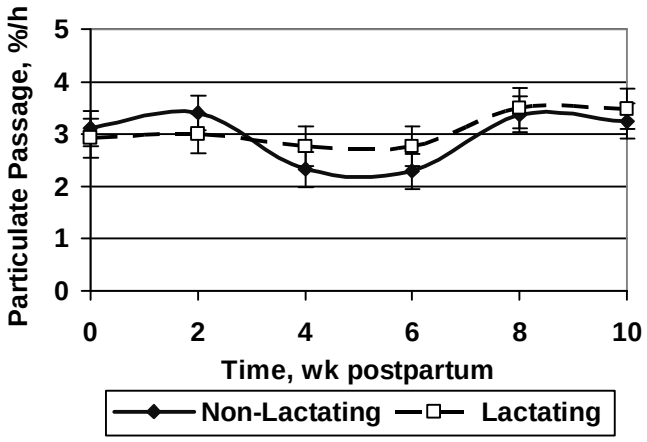

Figure 3. Ruminal particulate passage rate in primiparous beef heifers from parturition to 10 wk postpartum.

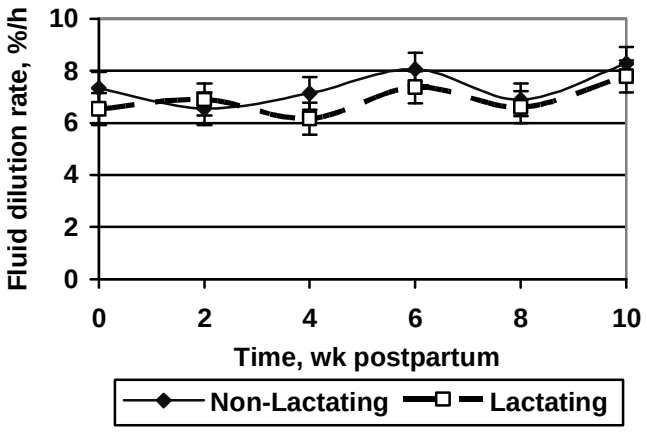

Figure 4. Ruminal fluid dilution rate in primiparous beef heifers from parturition to 10 wk postpartum.

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EFFECTS OF ADVANCING GESTATION ON DMI, DM DIGESTION, AND RUMINAL DYNAMICS OF PRIMIPAROUS BEEF HEIFERS FED LOW-QUALITY, WARM-SEASON GRASS HAY

D. R. Linden[1], K. C. Olson[1], J. R. Jaeger[2], L. W. Murray[3], D. E. Anderson[4], L. A. Pacheco[1], K. E. Holcomb[4], J. W. Bolte[2], N. A. Sproul[1], and M. D. Thomas[1],

[1] Dept. of Animal Sciences and Industry, Kansas State University, Manhattan, KS
[2] Agricultural Research Center - Hays, Kansas State University, Hays, KS
[3] Dept. of Statistics, Kansas State University, Manhattan, KS
[4] Dept. of Clinical Sciences, Kansas State University, Manhattan, KS

ABSTRACT: Ruminally-cannulated Angus X heifers (n = 12; initial BW = 525 ± 53 kg) were individually fed chopped, warm-season grass hay (6.5% CP and 36.8% ADF) *ad libitum* for 68 d prepartum. Seven heifers were pregnant and 5 heifers were non-pregnant. Total tract DM digestibility (DMD), ruminal VFA, ruminal NH_3, particulate passage, and fluid dilution rate were measured every 14 d. Both treatment groups increased (period main effect - $P < 0.01$) DMI during the course of the study; however, pregnant heifers ate less ($P = 0.05$) DM than non-pregnant heifers. Digestion of DM was similar ($P = 0.30$) between treatments and generally decreased (period main effect - $P < 0.01$) as intake increased. Ruminal NH_3 generally increased over time but the magnitude of the response was influenced by pregnancy status (treatment x period - $P = 0.04$). Total ruminal VFA concentration was similar ($P > 0.10$) between treatments at 10, 8, 6, and 4 weeks prior to parturition; however, pregnant heifers had less (treatment x period - $P < 0.01$) total ruminal VFA than non-pregnant heifers 2 weeks before parturition. Pregnant heifers had greater ($P < 0.03$) ruminal molar proportions of acetate and lesser ($P < 0.01$) ruminal molar proportions of butyrate and minor VFA than non-pregnant heifers. Molar proportion of propionate was similar ($P > 0.10$) between treatments 10 and 2 weeks before parturition. Conversely, pregnant heifers had greater (treatment x period - $P < 0.01$) molar proportions of propionate than non-pregnant heifers 8, 6, and 4 weeks before parturition. Particulate passage rate was similar between treatments ($P = 0.55$). Ruminal fluid dilution rate of pregnant heifers tended to be less ($P = 0.10$) than that of non-pregnant heifers. These data were interpreted to suggest that the changes to intake, passage rate, and ruminal fermentation that are characteristic of beef cows during late gestation may not occur in beef heifers.

Keywords: fermentation, gestation, heifers, intake, passage rate

Introduction

Late gestation is accompanied by elevated nutrient requirements in mature beef cows (NRC, 2000); however, forage dry matter intake (DMI) typically decreases during late pregnancy (Campling, 1966; Weston, 1988; Stanley et al., 1993; Allen, 1996; Scheaffer et al., 2001). The decrease in DMI is thought to be associated with reduction in ruminal volume caused by the rapid increase in fetal size during late gestation (Forbes, 1968). Little research has focused on changes that occur in DMI, DMD, and digesta passage rates in primiparous heifers; such information would improve prepartum nutritional management of this class of beef cattle. The objective of our study was to measure the effects of advancing gestation on DMI, DMD, passage rates, and ruminal fermentation by primiparous beef heifers.

Materials and Methods

Animals and Diet. All procedures used in the care, handling, and sampling of animals in our study were approved by the Kansas State University Institutional Animal Care and Use Committee. Commercial Angus heifers (n = 12; average initial BW 525 ± 53 kg) were housed indoors in individual tie-stalls (approximately 2 m x 1.2 m) in an environmentally-controlled barn (average temperature 25°C; average humidity 72%; 12 h light, 12 h dark) for an average of 68 d prepartum. Heifers were fed chopped (approximate particle length = 10 cm), warm-season grass hay (6.5 % CP, 36.8% ADF) *ad libitum* in individual feed bunks (87 cm long x 152 cm high x 85 cm wide). Hay was maintained in a covered barn before and after chopping. Daily hay refusals were weighed immediately prior to feeding at 0700. Water and trace-mineralized salt were available *ad libitum*.

Heifers were weighed every 14 d throughout the study; body condition scores (BCS) were assigned at the time body weights were measured as the average score given by 3 trained observers using a 9-point scale (1 = emaciated, 9 = obese; Neumann and Lusby, 1986). Treatment assignments were based on pregnancy status. Seven heifers began the study pregnant (average initial day of gestation = 213). These heifers were inseminated by transcervical artificial insemination after ovulation synchronization. Ovulation was synchronized using the 7-11 Co-Synch protocol as described by Eborn and Grieger (2007).

Data Collection. The prepartum period was divided into five 14-d data-collection periods. Total fecal output was estimated on d 9-12 using acid detergent-insoluble ash (ADIA) as an internal marker. Fecal grab samples were collected from the rectum every 4 h. Each day, sample collection times were advanced 1 h such that 1 sample had been collected at each h of the day (e.g., a total of 24 fecal samples per cow over four days) to account for diurnal

changes in composition. Fecal samples were dried for 72 h in a forced-air oven at 55°C to determine DM.

Ruminal fermentation and fluid dilution rates were characterized on d 13 of each collection period. Cobalt-EDTA was used as an external marker of the fluid phase of ruminal digesta (Uden et al. 1980); marker was pulse dosed at a rate of 6.5 g at 0800. Ruminal fluid samples were obtained from 3 areas of the ventral rumen just prior to marker dosing (0 h) and 4, 8, 12, 16, and 20 h after marker dosing. Ruminal fluid was strained through four layers of cheesecloth and separated into two aliquots: 10 mL were retained for Co analysis and 10 mL were combined with 2 mL of 25% (wt/vol) metaphosphoric acid for VFA and NH_3 analysis. Ruminal fluid aliquots were frozen immediately after collection.

Ruminoreticular fill and ruminal ADIA passage rates were measured on d 14 of each collection period. Fill was determined by complete manual evacuation of digesta (solid and liquid fractions) from the rumen and reticulum immediately prior to and 4 h after the daily feeding. Evacuations were performed via the ruminal fistula. Ruminoreticular contents were completely removed, weighed, and mixed; 4 subsamples of digesta were collected. After sampling, all contents were replaced via the ruminal fistula. Ruminal digesta DM was determined by drying samples in a 55°C forced-air oven for 72 h. Ruminal DM fill was estimated by multiplying ruminal digesta DM by the total weight of ruminal digesta. Ruminal fluid fill was estimated as the difference between total ruminal fill and ruminal DM fill. Ruminal particulate passage rate was determined by measuring the ratio of ADIA ingested to ADIA in ruminal digesta.

Laboratory Analyses. Forage, ort, fecal, and ruminal samples were dried for 72 h in a forced-air oven at 55°C and ground to pass through a 1-mm screen (Model 4 Wiley mill; Thomas Scientific, Swedesboro, NJ, USA). Crude protein was determined by combustion (AOCA, 1980). Neutral-detergent fiber and ADF were determined using an Ankom Fiber Analyzer (Ankom^{200}, Macedon, NY, USA).

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Statistical Analyses. Data were analyzed as a 6-period, 2-treatment completely random design using the MIXED procedure of SAS (SAS 9.1, 2009). Class variables included treatment, animal, and period. The model statement included terms for treatment, period, and treatment x period. Intake and DMD data were summarized as the means for each 2 wk period prior to the time of actual calving.

Data describing ruminal fermentation and passage rates were analyzed as a split-plot arrangement of a completely random design using the MIXED procedure of SAS. Whole plot effects included animal, period, and treatment. Subplot effects were time and treatment x time. Whole plot effects were tested using animal x period x treatment. Residual error was used to test subplot effects. When significant F-tests ($P \leq 0.05$) were observed, the method of least significant difference was used to partition treatment sums of squares.

Results and Discussion

Dry Matter Intake and Digestion. Both treatment groups increased (period main effect - $P < 0.01$) DMI during the course of the study (Figure 1); however, pregnant heifers ate less ($P = 0.05$) DM than non-pregnant heifers. Similar increases in DMI with advancing gestation have been reported in mature beef cows (Stanley et al., 1993). In contrast, Scheaffer et al. (2001) reported no difference in DMI between pregnant and non-pregnant beef heifers. Vanzant et al. (1991) reported greater intake by pregnant heifers at 55 d prepartum; however, there was no difference in DMI at 12 d prepartum between pregnant and non-pregnant heifers. Increases in DMI during early pregnancy can be attributed to heightened nutrient demand caused by the fetus and gravid uterus. Conversely, decreased DMI during late pregnancy is usually associated with the rapidly-growing fetal tissues creating a physical impingement on ruminal volume.

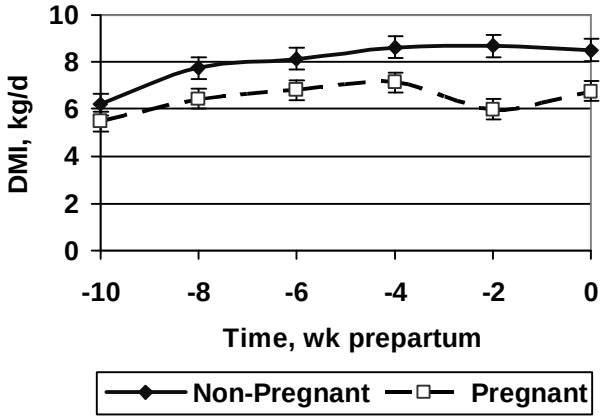

Figure 1. Dry matter intake (DMI) by pregnant first-calf beef heifers from 10 wk prepartum until parturition.

Dry matter digestibility (DMD) was similar ($P = 0.30$) between treatment groups but generally decreased (period main effect - $P < 0.01$) as intake increased (Figure 2). Hanks et al. (1993) reported no difference in DMD between pregnant and non-pregnant cows. Conversely, Vanzant et al. (1991) and Scheaffer et al. (2001) reported decreases in DMD in grazing and confined heifers, respectively. Increased DMI is expected to be associated with decreased DMD. Additionally, increased DMI is usually accompanied by more rapid fluid and particulate passage. Mean ruminal

retention time and the extent of DMD generally decrease under these conditions.

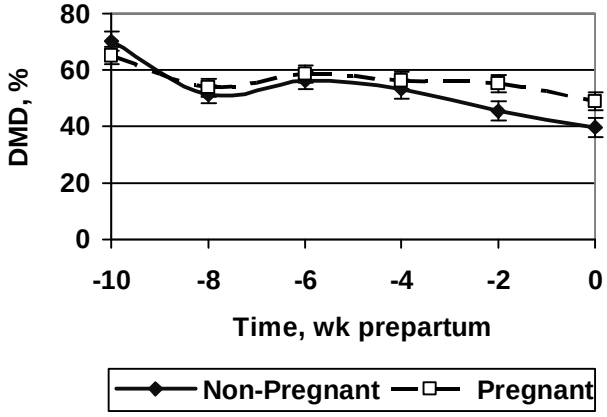

Figure 2. Dry matter digestion (DMD) by pregnant primiparous beef heifers from 10 wk prepartum until parturition.

Ruminal Fermentation. Ruminal NH_3 generally increased over time but the magnitude of the response was influenced by pregnancy status (treatment x period - $P = 0.04$; Table 1). Vanzant, et al. (1991) reported an increase in ruminal NH_3 early in pregnancy followed by a decrease in ruminal NH_3 during late pregnancy. Hanks et al. (1993) reported only minor temporal differences in ruminal NH_3 concentration between pregnant cows and non-pregnant cows. Conversely, Scheaffer, et al. (2001) reported a decrease in ruminal NH_3 with advancing gestation. A decrease in ruminal NH_3 concentration is often associated with an increase in ruminal passage rate or an increase in DMI. Moreover, Scheaffer et al. (2001) suggested that increased nutrient demand by the fetus may drive greater absorption of ruminal ammonia.

Total ruminal VFA concentration was similar ($P > 0.10$) between treatment groups at 10, 8, 6, 4, and 0 wk relative to parturition (Table 1). Pregnant heifers had less (treatment x period - $P < 0.01$) total ruminal VFA than non-pregnant heifers 2 wk before parturition. Scheaffer et al. (2001) and Vanzant et al. (1991) reported no differences in total VFA concentration between pregnant and non-pregnant beef heifers. In contrast, Hanks et al. (1993) reported decreased total VFA concentration in pregnant cows 68 d prepartum; however, non-pregnant cows had greater total VFA concentration 10 d prepartum.

The decrease in total VFA we observed 2 wk prepartum coincided with a decrease in DMI. Decreased DMI likely resulted in decreased substrate availability for ruminal microbes and a decrease in the products of fermentation.

Pregnant heifers had greater ($P < 0.03$) ruminal molar proportions of acetate and lesser ($P < 0.01$) ruminal molar proportions of butyrate and minor VFA when compared with non-pregnant heifers (Table 1). An increase in molar proportion of acetate is generally associated with a decrease in other VFA. Vanzant et al. (1991) and Scheaffer et al. (2001) reported no differences in ruminal molar proportion of acetate between pregnant and non-pregnant heifers. Similarly, Scheaffer et al. (2001) reported no difference in molar proportion of butyrate between pregnant and non-pregnant cows. .

Molar proportion of propionate was similar ($P > 0.10$) between treatments at 10 and 2 wk prepartum. Conversely, pregnant heifers had greater (treatment x period - $P < 0.01$) molar proportions of propionate than non-pregnant heifers 8, 6, and 4 weeks before parturition. Hanks et al. (1993) reported no difference in ruminal proportion of propionate between pregnant and non-pregnant beef cows.

Table 1. Total ruminal VFA and molar proportion of acetate, propionate, butyrate, and minor VFA* in primiparous beef heifers.

	Treatments				
Item	Pregnant	Non-Pregnant	SE	*P*	Effect
Ammonia, mM	1.20	1.18	0.2	0.04	Treatment x Period
Total VFA, mM	68.3	71.5	2.2	< 0.01	Treatment x Period
Acetate, % of total VFA	71.5	70.2	0.4	< 0.03	Treatment
Propionate, % of total VFA	36.8	36.4	0.2	< 0.01	Treatment x Period
Butyrate, % of total VFA	9.2	10.6	0.2	< 0.01	Treatment x Period
Minor VFA*, % of total VFA	2.2	2.6	0.1	< 0.01	Treatment

*Minor VFA included isobutyrate, valerate, and isovalerate

Passage Rate. Particulate passage rate was similar between treatments ($P = 0.55$; Figure 3), whereas ruminal fluid dilution rate of pregnant heifers tended to be less ($P =$ 0.10) than that of non-pregnant heifers (Figure 4). These data are contradictory to those reported by Weston (1983), Vanzant et al. (19910, and Hanks et al. (1993), in which

pregnant cattle had consistently greater passage rates compared with non-pregnant cattle. The tendency for decreased fluid dilution rate by pregnant heifers in our study was associated with decreased DMI by pregnant heifers relative to non-pregnant heifers.

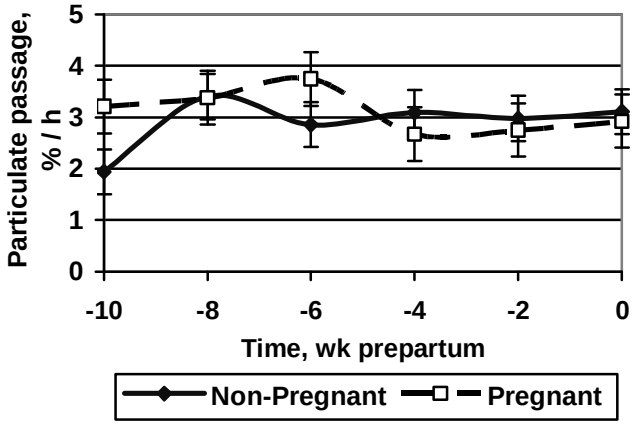

Figure 3. Ruminal particle passage rate in primiparous beef heifers from 10 wk prepartum until parturition.

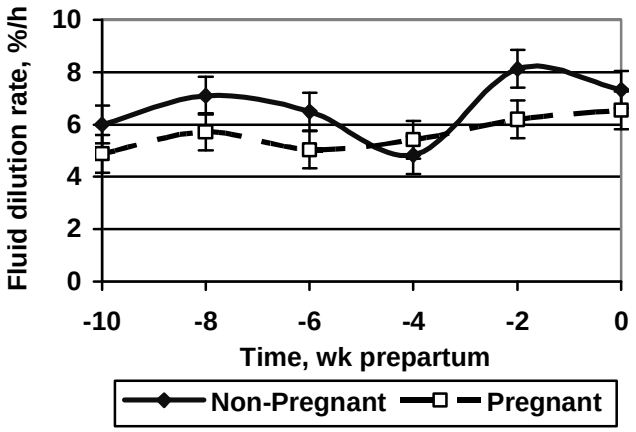

Figure 4. Ruminal fluid dilution rate in primiparous beef heifers from 10 wk prepartum until parturition.

Conclusions

These data were interpreted to suggest that the changes in intake, passage rate, and ruminal fermentation that are characteristic of beef cows during late gestation may not be as pronounced in beef heifers.

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EFFECT OF WINTER SUPPLEMENTATION STRATEGY ON GLUCOSE METABOLISM PRE- AND POSTPARTUM

F. W. Harrelson, S. L. Lodge-Ivey, S. H. Cox, R. L. Dunlap, J. T. Mulliniks, B. H. Carter, C. A. Löest, and M. K. Petersen

New Mexico State University, Las Cruces, NM

ABSTRACT: Forage quality and availability has been shown to directly influence circulating serum glucose concentrations as well as glucose utilization. The objective of our study was to determine the effects of winter protein supplementation on serum glucose half-life and clearance rate during late gestation (~52 d prepartum) and mid lactation (~ 54 d postpartum) as well as identify the effects of protein supplementation on mid lactation milk production. Cows (n = 8/treatment, 542 kg average BW) were supplemented until calving with 1) a control 36% CP (35% UIP) cottonseed meal based cube (CON), hand-fed at 454 g/d delivered 3d/wk ($16/45.4 kg), or 2) a self-fed 50% loose mineral and 50% fishmeal 33% CP (60% UIP) small supplement (SSP), formulated for a targeted consumption of 113g/d ($52/45.4 kg). After calving, cows were supplemented similarly (36% CP cube at 908 g/d offered 3 d/wk). No supplement × physiological state (prepartum or postpartum) interactions were observed ($P \geq 0.13$). Also, physiological state had no effect ($P > 0.24$) on glucose clearance. Glucose half-life was less ($P = 0.11$) for SSP than CON (52.5 vs. 80.5 min, respectively). Area under the curve (AUC) for glucose was also higher ($P = 0.06$) for CON compared to SSP (5403 vs. 3795, respectively). Insulin half-life was unaffected ($P = 0.61$) by supplement, though AUC for insulin was lower ($P = 0.05$) for SSP than CON. Milk production was not affected ($P = 0.28$) by winter supplementation strategy (7899 vs. 7164 ± 514 g; for CON and SSP respectively). Similarly, milk composition was unaffected by supplement strategy ($P \geq 0.20$). The results from this study suggest that the high UIP self-fed supplement increases glucose clearance rate compared to a traditional hand-fed supplement, and though not significant, supplemental UIP during the winter may have a carryover effect into lactation.

Keywords: beef cattle, glucose, protein, supplementation

INTRODUCTION

Diet quality and dam physiological state have been implicated in altering insulin sensitivity in beef cows (Bines and Hart, 1982; Endecott et al. 2004). Ruminal fermentation of dormant forage is characterized by predominately acetate production and a lower proportion of propionate (Cronje et al. 1991). As a result acetate may accumulate within the blood and along with increased concentration of ketones and free fatty acids which have been implicated in insulin resistance (Dresner et al., 1999; Tardiff et al., 2001). Winter supplementation is often a crucial part of managing spring calving herds on western rangeland. A major reason is due to the mature forage being low in protein and possibly energy (Krysl et al. 1987; Soder et al. 1995). Our objective was to investigate the effect of winter supplementation on serum glucose half-life and clearance rate during late gestation, mid lactation, as well as on mid lactation milk production.

MATERIALS AND METHODS

This study was conducted from January to May (2008) at the New Mexico State University Corona Range and Livestock Research Center, Corona, NM. The average elevation at the study site is 1,900 m. Annual precipitation averages 370 mm, with 70% of this precipitation occurring between May and October (Torell et al. 2008). This study is also a segment of a larger integrated systems approach research project evaluating maternal nutrition on calf health and performance and utilized cows discussed by Harrelson et al. (2009). All animal handling and experimental procedures were in accordance with the New Mexico State University Institutional Animal Care and Use Committee guidelines.

Sixteen Angus and Angus cross cows (542 ± 9 kg BW) were utilized prepartum; due to 1 cow losing a calf, and 1 cow calving late, fourteen of these cows (453 ± 9 kg BW) were utilized postpartum. Cows were 4.5 yr of age prepartum and 5 yr of age postpartum. Supplemental treatments consisted of 1) traditional 36% CP hand-fed supplement (CON) or 2) NMSU small self-fed supplement package (SSP).

The CON supplement was a cottonseed meal based range cube with 35% UIP. Composition was 57% cottonseed meal, 21% wheat middlings, 10% soybean meal, 9% molasses, 1.2% urea and fortified with trace minerals and vitamins. The CON supplement was fed at a rate of 454 g/d delivered 3 d/wk. The SSP supplement (NMSU small package supplement) was formulated to contain 33% CP (60% UIP), and composed of 50% fishmeal, 33 % minerals, and 17% salt.

Winter supplementation was ended two weeks prior to the expected start of parturition within the herd (mid-February). Supplementation strategy after parturition was similar for all cows (CON fed at 908 g/d fed 3 d/wk).

Glucose half-life and sensitivity to endogenous insulin was evaluated via a glucose tolerance test (GTT). Prepartum GTT was conducted approximately 52 d prior

to calving, while postpartum GTT was conducted approximately 54 d after calving. A 12-gauge hypodermic needle (Ideal Instruments, Schiller Park, IL) was utilized to puncture the jugular vein. One-half of 2.5m of tygon tubing (0.10 cm i.d., 0.18 cm o.d., Cole Parmer Instrument Co., Vernon Hills, IL) was threaded through the needle and into the jugular vein. The remaining portion was secured to the neck and down the middle of the back of each cow via tape. A blunt 18-gauge needle (Salvan Dental Specialties, Charlotte, NC) was inserted in the end of the catheter and a 10-mL syringe was used as the tubing end cap. Catheters were placed into each animal the morning of the GTT. A 50% dextrose solution was infused at 0.25 ml/kg BW via the indwelling jugular catheter. Blood samples were collected at -1, 0, 3, 6, 9, 12, 15, 20, 40, 60, 80, 100, 120, 140, 160, and 180 min relative to dextrose infusion. Catheters were flushed with 10 mL of a 9% saline solution post dextrose infusion as well as immediately before and after each collection time. Sample collection time -1 was collected before dextrose infusion and time 0 immediately after infusion. Ten-milliliter blood samples were collected at each time point and placed in Corvac serum separator tubes. Blood samples were centrifuged at 2000 × g at 4°C for 20 min. Serum was stored in plastic vials at -20°C until glucose and insulin analyses were conducted. Glucose was analyzed with a commercial kit (enzymatic endpoint, Thermo Electron Corp., Waltham, MA). Insulin was analyzed by solid-phase RIA (DCP kit, Diagnostic Products Corp., Los Angeles, CA) as reported by Reimers et al. (1982). Intra- and inter-assay CV for both insulin and glucose were < 10%. Serum glucose and insulin areas under the curve (AUC) were calculated using trapezoidal summation. Glucose half-life was estimated by determining the time required for a 50% decrease in peak serum concentrations.

Twenty cows, those utilized for GTT as well as an additional 6 cows of similar d in milk, were machine milked approximately 50d postpartum. Milking was conducted one week prior to GTT. Milking procedures were a modified weigh-suckle-weigh technique as described by Appeddu et al. (1997). Milk weights were recorded in order to determine 24hr milk production. Milk samples were analyzed for lactose, butterfat, protein, and solids non-fat by Silliker Inc. (Artesia, NM).

Data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inc., Cary, NC) with cow as the experimental unit using the Kenward-Roger degrees of freedom method. Supplement strategy, physiological state (pre or postpartum) and their interaction were utilized in the model as a fixed effects. Calf birth weight was also included in the model as a covariate.

RESULTS AND DISCUSSION

The effects of supplementation strategy on glucose kinetics and milk production are presented in Table 1. Supplement affected the glucose half-life (P = 0.11), glucose AUC (P = 0.06) and insulin AUC (P = 0.05). Glucose half-life was shorter for SSP than CON (52.6 vs. 80.5 min); also, glucose and insulin AUC were less for SSP compared to CON. Insulin half-life (P = 0.61) and the insulin to glucose ratio (P = 0.71) were unaffected by supplement. The results of our study agree with Waterman et al. (2006), which showed that feeding supplements with higher UIP decreased glucose half-life and therefore resulted in cows being less insulin resistant. No significant effects (P > 0.20) of supplement on milk production were observed. Mulliniks et al. (2008) also found no differences in milk production due to increasing insulin sensitivity. Cows receiving CON during the winter trended toward producing more milk, and had higher levels of milk constituents, which would be expected due to the higher glucose half-life thereby glucose to be cleared slower and making more available to the udder for milk production. Since glucose is converted to lactose and becoming the osmotic factor necessary for fluid milk production (Vilotte 2002). Since glucose clearance rates were higher for CON, more glucose would be in circulation and available to be utilized for milk production and composition. These results are in disagreement with Van Saun et al (1993), who found that feeding higher UIP diets resulted in higher milk protein production in dairy cattle.

Table 2 illustrates the effect of physiological state on glucose metabolism. No significant effects were observed for glucose kinetics when comparing the prepartum to postpartum challenges. Though not significantly different, both glucose and insulin half-lives trended to be longer prepartum than postpartum indicating that potentially during late gestation these cows may be slightly more insulin resistant than while lactating. These findings are similar to those of Waterman et al. (2007) and may also be attributed forage quality as the prepartum GTT was conducted during January when forage is dormant. The postpartum GTT's were conducted in late April and early May, at which time the forage quality may be beginning to improve as moisture and temperature rise.

The effects of supplement and physiological state are presented in Table 3. Glucose AUC tended (P = 0.14) tended to be influenced by an interaction between supplement and physiological state, with those receiving SSP having higher values prepartum while those receiving CON had higher values postpartum. No other measure of glucose metabolism was affected by the interaction of supplement and physiological state. All glucose half-lives were at least 1.5 or more times slower than the normal 35 min described by Kaneko (1997).

Increasing glucose clearance rates limits the buildup of both ketones and non-esterified fatty acids within the blood. Fewer of these compounds results in cows which have a more neutral nutrient balance and are less susceptible to ketosis or fatty liver.

IMPLICATIONS

Supplementation with a high UIP self-fed supplement may be an effective way to decrease insulin resistance and therefore allow for increased animal production during late gestation and these effects may potentially be transferred into mid lactation which was an unexpected result.

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Table 1. Effect of supplementation strategy on glucose metabolism and milk production.

Item	CON	SSP	SEM	*P*-value[1]
Glucose Metabolism				
Glucose Half-life, min	80.5	52.6	12.1	0.11
Glucose AUC	5402	3794	580	0.06
Insulin Half-life, min	27.9	25.3	3.6	0.61
Insulin AUC	67.9	50.1	6.1	0.05
Insulin:Glucose ratio	0.015	0.016	0.003	0.71
Milk Production				
24hr Production, g	7898	7164	514	0.28
Butterfat, g	269	221	32	0.26
Lactose, g	395	355	25	0.23
Protein, g	204	184	17	0.41
Solid non-fat, g	670	604	46	0.28

[1]Protected F-statistic for the effect of supplement strategy.

Table 2. Effect of physiological state on glucose metabolism[1].

Item	Pre	Post	SEM	*P*-value[2]
Glucose Half-life, min	71.7	61.43	12.2	0.55
Glucose AUC	4113	5084	581	0.24
Insulin Half-life, min	29.0	24.2	3.6	0.34
Insulin AUC	54.8	63.2	6.1	0.33
Insulin:Glucose ratio	0.018	0.014	0.003	0.29

[1]Pre = Prepartum; Post = Postpartum.
[2]Protected F-statistic for the effect of physiological state.

Table 3. Effect of physiological state and supplement on glucose metabolism.

	Physiological State			
Item	Prepartum	Postpartum	SEM	*P*-value[1]
Traditional Supplement (CON)				
Glucose Half-life, min	87.7	73.3	18.5	0.81
Glucose AUC	4299	6505	885	0.14
Insulin Half-life, min	29.2	26.5	5.5	0.68
Insulin AUC	62.8	73.0	9.3	0.83
Insulin:Glucose ratio	0.018	0.012	0.004	0.63
Small Supplement (SSP)				
Glucose Half-life, min	55.6	49.5	16.9	0.81
Glucose AUC	3927	3662	810	0.14
Insulin Half-life, min	28.8	21.8	5.0	0.68
Insulin AUC	46.8	53.4	8.5	0.83
Insulin:Glucose ratio	0.017	0.015	0.004	0.63

[1]Protected F-statistic for the effect of supplement×physiological state.

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Supplementing wet distillers grains mixed with low quality forage to grazing cow calf pairs.

B.L. Nuttelman*, W.A. Griffin, T.J. Klopfenstein, W.H. Schacht, L.A. Stalker, J.A. Musgrave, and J.D. Volesky
University of Nebraska-Lincoln

ABSTRACT: Two consecutive summer grazing studies were conducted to quantify the effect of supplementing cows with wet distillers grains (WDGS) mixed with low quality forage on 1) grazed forage intake and 2) cow and calf performance. During exp. 1, twenty-four three year old lactating cows rotationally grazed for 56 d and were assigned to one of three treatments: 1) the recommended stocking rate of 1.48 AUM/ha with no supplementation (CON1), 2) double the recommended stocking rate (2.96 AUM/ha) and supplemented 6.64 kg/hd daily of 45% grass hay and 55% WDGS (DM) to replace 50% of estimated total intake (SUP), and 3) grazing at 2.96 AUM/ha with no supplementation (2X). In exp. 2, forty two-year old lactating cows rotationally grazed the same paddocks as in exp. 1 for 56 d and were assigned to one of four treatments grazing at: 1) recommended stocking rate (1.48 AUM/ha) with no supplementation (CON2), or double the recommended stocking rate and receiving 5.8 kg/hd daily of wheat straw and WDGS mixed at: 2) 70:30 (LOW), 3) 60:40 (MED), or 4) 50:50 (HIGH). Supplemented groups were fed at 50% of estimated total intake. For both studies forage utilization was determined by clipping twenty, 1-m^2 quadrats pre- and post-grazing. For exp. 1, SUP cows had higher ADG (0.25 kg/d; $P < 0.01$) than CON1 and 2X (-0.45 and –0.52 kg/d, respectively). Calf daily gain was higher for SUP than for CON1 and 2X (1.07, 0.82, and 0.75 kg/d; $P < 0.01$). Forage utilization (% standing green) for CON1 was 51.1 and 68.0% less than SUPP and 2X, respectively ($P < 0.01$). For exp. 2, HIGH cows were the heaviest at the end of the study ($P = 0.04$). Forage utilization was less for CON2 than for HIGH or MED (34.4, 45.9, and 44.3%, respectively; $P < 0.02$), but was similar for CON and LOW (38.4%; $P = 0.18$). Grazing cows supplemented wheat straw and 45% or greater WDGS gained more weight. Grazing intake was reduced the most when wheat straw was 70% of the mix.

KEYWORDS: Grazing, Forage Intake, Supplementation, Lactating Cows

Introduction

Recent research has been successful in mixing and storing WDGS mixed with low quality forages to extend the shelf life of the WDGS (Adams et al., 2008), and in feeding this mixture to growing calves (Nuttelman et al., 2008). Storing WDGS for extended lengths of time can be beneficial to cow/calf producers. Cattle consuming high forage diets eat to a constant fill as determined by NDF (Van Soest, 1965). Mixing WDGS with low-quality forage increases the palatability of the forage, and the additional bulk from the forage can potentially reduce grazed forage intake by supplying fill. Therefore, two consecutive summer grazing studies were conducted to determine the effect of supplementing cows with wet distillers grains (WDGS) that had previously been mixed and stored with low quality forage on 1) grazed forage intake and 2) cow and calf performance.

Materials and Methods

For both studies, the experiment was replicated over two blocks based on location (east and west) due to variation in species composition and topography. Standing crop and forage utilization was determined by clipping 20 1-m^2 quadrats both pre- and post-grazing, and quadrats were sorted by live grass, forbs, standing dead, and litter and then dried and weighed to determine forage availability. Forage disappearance (DIS) was determined for each paddock by calculating the difference in pre-graze forage allowance and the amount of forage that remained following the grazing period. Cow/calf pairs were limit fed meadow hay at 2% of BW for five days prior to and at the conclusion of the grazing period to eliminate

variation due to gut fill. At the conclusion of both limit feeding periods, cows and calves were individually weighed for three consecutive days, and the average of the weights were used as the initial and ending BW. Cattle that received supplement (MIX) were supplemented at 50% of their estimated daily intake, and were fed in feed bunks located outside of the grazing paddock to eliminate trampling of forage around the feeding site.

Exp. 1

Twenty-four three-year old, non-gestating, lactating cows with spring born calves at side grazed their assigned paddocks for 56-d during the summer. Paddocks were 1-ha and were assigned randomly to one of three treatments that consisted of: 1) the recommended stocking rate of 1.48 AUM/ha with no supplementation (CON1), 2) double the recommended stocking rate (2.96 AUM/ha) and supplemented 6.64 kg/hd daily of 55% grass hay and 45% WDGS (DM) to replace 50% of estimated total intake (SUP), and 3) grazing at 2.96 AUM/ha with no supplementation (2X). The paddocks that were assigned to the increased stocking rate were divided in half, and cattle were only allowed to graze one-half of the paddocks per grazing period. Cattle were rotated through seven paddocks, and the days of grazing for each paddock were adjusted prior to initiation of the trial to account for stage of plant growth.

Exp. 2

The year following Exp. 1, a second study of similar design was conducted in the same paddocks to compare different mixes of WDGS and wheat straw. Wheat straw was chosen to serve as a source of lower quality forage that contained more NDF than the grass hay used in the previous year. Wheat straw was mixed with WDGS at three different levels consisting of 50:50, 40:60, and 30:70 WDGS:wheat straw on a DM basis, and was stored in an ag bag thirty days prior to initiation of the trial. Water was added during mixing to the two lower levels of WDGS until moisture was equal to the high level of WDGS.

Twenty paddocks were arranged by the previous year's usage and grazing order, and then assigned to one of four treatments: 1) Control (CON2), 2) 50:50 WDGS:wheat straw supplement (HIGH), 3) 40:60 WDGS:wheat straw supplement (MEE), or 4) 30:70 WDGS:wheat straw supplement (LOW). The hypothesis was that the additional straw would provide more bulk and result in a larger replacement rate of grazed forage due to a fill effect. The CON2 was stocked at the recommended stocking rate of 1.48 AUM/ha, and the paddocks assigned to treatments receiving supplementation were grazed at double the recommended stocking rate (2.96 AUM/ha). The paddocks grazed at double the stocking rate were divided in half to decrease the amount of area allowed for grazing. Forty two-year old lactating cows with spring born calves at side were utilized and assigned to paddock rotation. Cattle within block grazed the assigned paddock in the experimental pastures for seven days. When cattle were not grazing the experimental pastures, they were moved to a pasture of similar forage species composition and managed separately. They continued to be supplemented with the mix to measure differences in animal performance.

Results and Discussion

Exp. 1

Initial BW (Table 1) was not different for the individual cow, or the individual calves ($P > 0.89$). Final BW was not different ($P > 0.13$), but SUP cows and calves were numerically heavier than non-supplemented counter parts. Cows receiving SUP gained 0.70 and 0.77 kg more per d ($P < 0.01$) than CON1 and 2X, respectively. Non-supplemented calves gained 0.25 and 0.32 kg per d less than supplemented calves ($P < 0.01$). The extra gain for supplemented calves can be a result of increased milk production from the dam or the direct consumption of the MIX by the calves, or a combination of the two. The calves were at the bunk and appeared to be eating each d, however it is not possible to determine the actual amount of MIX that the calves consumed.

Percent utilization was determined by dividing the amount of forage that disappeared during the grazing period by the amount of available forage prior to grazing. The double stocked treatments had higher percent utilization than CON1 (33.1%; $P < 0.01$). There was no difference ($P = 0.15$) between SUP (52.0%) and 2X (57.8%) treatments. Grazed forage disappearance was determined by dividing the amount of forage that disappeared by the number of cow/calf pairs and the number of days each paddock was grazed. There were no differences ($P = 0.44$) for DIS between CON1, SUP, or 2X (12.6, 11.1, and 11.6 kg, respectively).

Exp. 2

Initial BW (Table 2) was not different among treatments for Exp. 2 ($P > 0.27$). Ending

BW was affected by supplementation ($P = 0.04$). Cattle receiving HIGH supplement were heavier at the conclusion of the study when compared to CON2, LOW, and MED (944, 875, 899, and 906 kg, respectively). Cattle on MED treatment tended ($P = 0.09$) to be heavier than CON2 at the end of the study. Average daily gain tended ($P = 0.06$) to be different between cows. Calf ending BW ($P = 0.63$) and ADG ($P = 0.46$) were different.

Cattle on CON2 had significantly less utilization than HIGH and MED (34.4, 46.0, and 44.3 %, respectively; $P = 0.02$). However, CON2 (34.4%) and LOW (38.4%) were not different ($P = 0.27$). The CON2 cattle had greater DIS than supplemented treatments ($P < 0.01$), but there was no difference for DIS for HIGH, MED, and LOW treatments ($P > 0.11$). For the supplemented treatments, the amount of forage that disappeared during the grazing period in addition to the DMI of the supplement was similar to the DIS of the CON2 ($P = 0.12$). This suggests that the supplemented cattle had similar DMI as the CON2 cattle. The amount of NDF consumed (not reported) from the grazed forage intake for the CON2 was compared to the NDF intake of the treatments that received supplement. The combined NDF intake from the grazed forage intake and the supplement was similar to the CON2 NDF intake (7.1 and 7.0 kg NDF/d; $P = 0.89$). This suggests the fibrous nature of most range diets limit VDMI by physical conditions and agrees with Balch and Campling (1962) and Ellis (1978) who reported the capacity of the reticulo-rumen limits voluntary intake by rate of disappearance of digesta from this organ. Similarly, Van Soest (1965) reported NDF to be the most influential chemical measure in relation to regulating VDMI.

In conclusion, cattle receiving higher levels of WDGS in the supplement resulted in improved performance during the grazing season. Supplementing low-quality forage mixed with WDGS can reduce grazed-forage intake. The percent NDF of the low quality forage appeared to determine the replacement rate of grazed forage intake by supplying a fill affect.

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Table 1. Exp. 1 Animal Performance and Grazing Results.

	Treatment				
	CON1[a]	SUP[b]	2X[c]	SE	*P*-Value
Initial, kg					
Cow	461	461	459	14	0.99
Calf	115	112	112	4	0.89
ADG, kg/					
Cow	-0.45[a]	0.25[b]	-0.52[a]	0.03	< 0.01
Calf	0.82[a]	1.07[b]	0.75[a]	0.01	< 0.01
% Utilization	33.1[a]	52.0[b]	57.8[b]	0.1	< 0.01
DIS kg/d[d]					
Green	12.6	11.1	11.6		
MIX	--	6.7	--		

[a] Cattle grazed at recommended stocking rate and received no supplementation.
[b] Cattle grazed at double the recommended stocking rate and received 50% of estimated daily intake of 45:55 WDGS:Wheat straw mixture.
[c] Cattle grazed at double the recommended stocking rate and received no supplementation.
[d] Calculated by dividing total amount of grazed forage disappearance by number of cow/calf pairs and number of grazing days.

Table 2. Exp 2 Animal Performance and Grazing Results.

	Treatment					
	CON2[c]	LOW[de]	MED[df]	HIGH[dg]	SE	*P*-Value
Initial, kg						
Cow	399	400	405	405	9	0.63
Calf	125	127	121	121	7	0.53
ADG, kg/d						
Cow	-0.03	0.13	0.11	0.42	0.14	0.06
Calf	0.89	0.90	0.89	0.99	0.09	0.46
% Utilization	34.4[a]	38.4[ab]	44.3[b]	46.0[b]	0.3	0.01
DIS, kg/d[h]						
Green	11.5[a]	6.1[b]	7.5[b]	7.4[b]	0.6	< 0.01
MIX	--[a]	5.8[b]	5.7[b]	5.9[b]	0.1	< 0.01

[a,b] Means with different superscripts differ (*P* –Value < 0.05).
[c] Cattle grazed at the recommended stocking rate.
[d] Cattle grazed at double the recommended stocking rate, and received 50% supplement of estimated daily intake.
[e] Cattle supplemented with 70:30 Wheat straw:WDGS mixture.
[f] Cattle were supplemented with 60:40 Wheat straw:WDGS mixture.
[g] Cattle were supplemented with 50:50 Wheat straw:WDGS mixture.
[h] Calculated by dividing total amount of grazed forage disappearance by number of cow/calf pairs and number of grazing days

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USE OF A SELF-FED, SMALL-PACKAGE PROTEIN SUPPLEMENT FOR BEEF COWS POST-WEANING[1]

R. L. Endecott[2] and R. C. Waterman[3]

[2]Montana State University, Miles City, MT
[3]USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT

ABSTRACT: A 2-year supplementation study conducted at Miles City, MT from mid-October to mid-December in 2007 and 2008 evaluated responses of beef cows (n = 141 in 2007, n = 138 in 2008; avg BW = 546 ± 5.2 kg) grazing dormant native range (8.8% CP, 64% NDF, 71% IVDMD) to two different supplementation strategies. Each year, cows were stratified by age and weight at weaning and then assigned to one of two supplements: 1) self-fed loose mineral mix (**MIN**) or 2) self-fed mineral plus high-bypass protein sources (**MIN+PRO**; 50% mineral mix, 25% feather meal, 25% fish meal). Target intakes were 70 g/d for MIN and 140 g/d for MIN+PRO. Cows were weighed and hip height and girth measurements were taken at the beginning and end of the 60-d studies. Weight-to-height and weight-to-girth ratio changes were calculated. Data were analyzed with supplement, cow age (2, 3, and 4+), year, and their interactions in the model. In 2007, cows fed MIN consumed 28 g/d and MIN+PRO cows consumed 93 g/d, which was lower than the target amount for both supplements. In 2008, MIN cows again failed to consume the target amount (13 g/d), while MIN+PRO cows consumed just over target amount (160 g/d). Cows lost similar (P = 0.70) amounts of weight during the study regardless of supplement treatment (-22 and -25 ± 5 kg for MIN and MIN+PRO, respectively). Likewise, weight-to-height ratio change (-0.25 and -0.25 ± 0.04) and weight-to-girth ratio change (-0.10 and -0.12 ± 0.02) were similar ($P \geq$ 0.60) for MIN and MIN+PRO cows, respectively. Year × cow age interactions ($P \leq$ 0.08) were observed for weight change and weight-to-height ratio change. Two- and 3-yr-old cows lost less weight in 2008 than in 2007, while mature cows lost similar amounts of weight in both years. All cows exhibited less change in weight-to-height ratio in 2008 compared to 2007, with the difference between years most pronounced in younger cows. Protein supplementation at this level did not impact cow performance; however, forage quality was higher than expected, which may have contributed to the lack of response to supplementation with the mineral-protein mix.

Key Words: Beef Cows, Post-Weaning, Protein Supplementation

Introduction

Low amounts of supplemental protein, particularly from sources high in ruminally undegradable protein (**RUP**), may enhance nitrogen utilization efficiency (Sawyer et al., 1998; Coomer et al., 1993). Freetly and Nienaber (1998) suggested that nutrient restriction also increases the efficiency of nitrogen utilization in cows. A supplement composed of small quantities of high-RUP (> 70% of CP as RUP) ingredients combined with salt and minerals was demonstrated to maintain ruminal function with low quality forage diets (Sawyer et al., 2000) and was consumed in controlled and consistent patterns by cows grazing desert range (Stalker et al., 2002). In a 3-year field study in central New Mexico, gestating cows consuming a small-package, self-fed supplement (25% feather meal, 25% blood meal, and 50% mineral mix; < 250 g/d consumption) maintained BW and BCS during late fall and early winter, and had similar performance to cows hand-fed an oilseed-based supplement at > 454 g/d (Sawyer et al., 2005). The objective of this study was to evaluate the effectiveness of a self-fed small-package supplement for maintaining BW of post-weaning beef cows grazing native range in the Northern Great Plains.

Materials and Methods

A 2-yr supplementation study was conducted at the Fort Keogh Livestock and Range Research Laboratory near Miles City, MT from mid-October to mid-December in 2007 and 2008. At this location, the potential natural vegetation is a grama-needlegrass-wheatgrass (*Bouteloua-Hesperostipa-Pascopyron*) mixed grass dominant. Average annual rainfall is 343 mm, with the majority occurring during the mid-April to mid-September growing season. Average precipitation compared to 2007 and 2008 precipitation patterns by month is presented in Figure 1.

Each year, Hereford and Hereford-cross beef cows (n = 141 in 2007, n = 138 in 2008; avg BW = 546 ± 5.2 kg; ages 2 through 11 yr; ~135 d gestation) were stratified by age and BW at weaning and then randomly assigned to one of four pastures. Supplement treatments (n = 2) were then randomly assigned to each pasture resulting in 2 pastures per supplement treatment. Treatments consisted of: 1) self-fed loose mineral mix (**MIN**; Table 1) or 2) self-fed mineral plus high-bypass protein sources (**MIN+PRO**). The MIN+PRO supplement was formulated to contain 35% CP and was composed of 50% mineral mix, 25% feather meal, and 25% fish meal. The mineral portion of MIN+PRO was designed to provide the same level of mineral intake as cows receiving MIN. Target intakes were 70 g/d for MIN

[1]USDA-ARS, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Research was conducted under a cooperative agreement between USDA-ARS and the Montana Agric. Exp. Stn. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana Agric. Exp. Stn., or the authors and does not imply its approval to the exclusion of other products that also may be suitable. The authors gratefully acknowledge W. Kelly, S. Reil, and B. Shipp for their technical assistance.

and 140 g/d for MIN+PRO. Cows were weighed and hip height and girth measurements were taken at the beginning and end of the study. Weight-to-height and weight-to-girth ratio changes were calculated.

Diet quality was estimated from ruminal extrusa. Extrusa samples were collected via ruminal evacuation techniques (Lesperance et al., 1960) at the beginning (mid-October) and end (mid-December) of the experiment in each year. Mature ruminally-cannulated cows (n = 2 per pasture) that grazed in common with experimental cows were used for all diet sample collections. Collected extrusa samples were lyophilized, ground to pass a 1-mm screen and stored until analysis for DM, OM (AOAC, 1990), and NDF (Goering and Van Soest, 1970). For CP analysis, sub-samples of ground extrusa were placed in glass square-bottom jars with metal rod inserts and dried in a 60°C oven. Upon removal from a drying oven, jars were capped with lids and subsequently placed on a roller grinder for 24 h (Mortenson, 2003). Nitrogen was determined by combustion techniques using a C-N analyzer (CE Elantech, Inc., Lakewood, NJ). Nitrogen values were multiplied by 6.25 to obtain CP.

At 0700 on the day of in vitro analyses, rumen extrusa (1/3 solids and 2/3 liquor) were collected at the interface of the forage mat and liquid fraction from 2 ruminally-cannulated cows on alfalfa hay diets and placed into a Dewar flask (Nalgene 4150-200, StevenJo & Steph, Rochester, NY) that had been incubated to 39°C for 24 h. Rumen extrusa was immediately transported in the Dewar flask to the laboratory at Fort Keogh and forage (solids) were placed into a blender for 30 s. Once extrusa was blended, solids and remaining rumen liquor were strained through 4 layers of cheesecloth into a 6-L Erlenmeyer flask that had been pre-warmed in a 39°C water bath under continuous CO_2 flushing. Next, 500 mL of rumen liquor was measured out into a graduated cylinder and was then combined with 500 mL of pre-made phosphate buffer [70.8% Na_2HPO_4 and 29.2% KH_2PO_4; Menke et al., (1979)] and McDougal's buffer (Tilley and Terry, 1963) already in vessels of a DAISY^{II} apparatus (ANKOM Technology Corp., Fairport, NY) maintained at 39°C. Vessels also contained samples [250 mg of sample/bag (F57; 5 × 5.55 cm^2, ANKOM Technology Corp, Fairport, NY)]. Vessels were purged with CO_2 for 30 s and a lid was secured onto the jar and immediately placed back into the DAISY^{II} apparatus (process was repeated for each of two vessels). Samples were then subjected to in vitro incubation for 48 h at 39°C. At the end of 48 h, incubation bags containing samples were removed and rinsed under reverse-osmosis water until effluent was clear. In vitro dry matter disappearance (IVDMD) was calculated as the DM which disappeared from the initial DM weight inserted into the bag.

Data were analyzed as a completely randomized design by analysis of variance using the MIXED procedure of SAS (SAS Institute, Cary, NC) with pasture as the experimental unit. The model included cow age (2-yr-old, 3-yr-old, or 4-yr-old and older), supplement, and their interaction as fixed effects and pasture within year by treatment as the random effect.

Results and Discussion

In 2007, cows fed MIN consumed 28 g/d and MIN+PRO cows consumed 93 g/d, which was lower than the target amount for both supplements (70 and 140 g/d, respectively). In 2008, MIN cows again failed to consume the target amount (13 g/d), while MIN+PRO cows consumed just over target amount (160 g/d). Sawyer et al. (2005) fed a supplement similar to MIN+PRO (containing blood meal instead of fish meal) to prepartum cows, who consumed an average 230 g/d over a three-year study. Stalker et al. (2002) reported intake of 128 g/d of a self-fed supplement similar to MIN+PRO.

No supplement × year, supplement × cow age, or supplement × cow age × year interactions were observed. ($P \geq 0.21$). Improved intake of MIN+PRO in 2008 did not result in a change in animal performance, and cows lost similar amounts of weight during the study regardless of supplement treatment ($P = 0.67$; -22 and -25 ± 4 kg for MIN and MIN+PRO, respectively). Likewise, weight-to-height ratio change and weight-to-girth ratio change were similar for MIN and MIN+PRO cows ($P \geq 0.56$; weight-to-height: -0.25 and -0.25 ± 0.03; weight-to-girth: -0.10 and -0.12 ± 0.02 for MIN and MIN+PRO, respectively). Sawyer et al. (2005) reported that gestating cows fed a small-package (< 250 g/d) self-fed protein supplement maintained weight and body condition during late winter, while cows that were self-fed loose mineral supplement lost weight. These researchers reported that cows on the mineral-only treatment were also fed 454 g/d oilseed-based supplement during adverse weather.

Cow age × year interactions were observed for weight change and weight-to-height ratio change ($P \leq 0.08$; Table 3). Two- and 3-yr-old cows lost less weight in 2008 than in 2007, while mature cows lost similar amounts of weight in both years. A similar pattern was observed for weight to height ratio.

Forage quality and quantity are important factors that influence domestic rangeland livestock production. Extrusa CP concentrations were similar between mid-October and mid-December each year ($P = 0.68$; 8.6 and 8.9 ± 0.5% CP for October and December, respectively), but were higher overall in 2008 than 2007 ($P = 0.04$; 9.6 and 8.0 ± 0.5% CP for 2008 and 2007, respectively). Higher CP concentrations in 2008 may be due to higher-than-average fall precipitation during October 2008 (Figure 1), coupled with unusually warm average temperatures during October and November (NOAA, 2008; data not shown). Extrusa NDF concentrations and IVDMD were each similar ($P \geq 0.61$) regardless of year or sampling time. These results may have been influenced by low stocking rates used in the experiment, which allowed animals to select diets of higher quality throughout the 60-d study each year. Even though forage quality characteristics indicate adequate nutrient supply for maintenance of gestating cows, cows still lost weight during the experiment. This might be partially explained by rapid temperature changes and inclement weather near the end of the 60-d study in both years. Both 2007 and 2008 were characterized by unseasonably warm temperatures early in the study period, followed by a rapid change to unseasonably cold temperatures during the last 2 wk of each 60-d period

(NOAA, 2007, 2008; data not shown). Adams et al. (1986) found reduced grazing activity and forage intake of grazing beef cows as minimum daily temperatures decreased. Adverse weather conditions experienced by the cows near the end of each 60-d study may have resulted in decreased grazing activity, forage intake, and subsequent weight loss.

Implications

Strategic protein supplementation with a small-package, self-fed supplement did not impact cow performance. Target intakes of the mineral-protein mix were only achieved in 1 of 2 years, which may have contributed to the lack of response. Cows were able to select a high quality diet during both years due to low stocking rates. Cow body weight loss despite diet quality characteristics indicating adequate nutrient supply may be due to rapid temperature drops and inclement weather near the end of the experiment in both years. Further research identifying liming nutrients in range forages and the use of strategic small-package supplementation may be beneficial to optimize range livestock production.

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Table 1. Composition of self-fed loose mineral supplement

Item	%
Calcium	12.0
Phosphorus	12.0
Salt	27.0
Sodium	10.0
Magnesium	1.5
Potassium	0.1
	ppm
Copper	1,200
Manganese	4,000
Iodine	100
Selenium	25
Zinc	2,500
	IU/kg
Vitamin A	330,000
Vitamin D	39,600
Vitamin E	220

Table 2. Crude protein and neutral detergent fiber concentration and in vitro dry matter disappearance of rumen extrusa samples at the start (mid-October) and end (mid-December) of supplementation from experimental pastures.

	Extrusa Collection		
Item	Supplementation Start	Supplementation End	SE
CP %, DM basis			
2007	8.4	7.6	0.8
2008	8.9	10.3	0.8
NDF %, DM basis			
2007	63.3	63.7	1.5
2008	64.5	64.5	1.5
IVDMD, %			
2007	69.4	72.8	2.4
2008	72.2	67.7	2.4

Table 3. Cow age × year interactions for weight change, weight-to-height ratio change and weight-to-girth ratio change.

	Cow Age						
Item	2	SE	3	SE	≥ 4	SE	Interaction *P*-value
Weight change, kg							
2007	-29^{ax}	5	-32^{ax}	6	-27^{ax}	4	0.03
2008	-15^{ay}	5	-13^{ay}	6	-26^{bx}	4	
Weight-to-height ratio change							
2007	-0.32^{ax}	0.04	-0.32^{ax}	0.05	-0.32^{ax}	0.03	0.08
2008	-0.17^{ay}	0.04	-0.13^{ay}	0.05	-0.25^{bx}	0.03	

[a,b] Means in rows with different superscripts differ ($P < 0.10$).
[x,y] Means in columns with different superscripts differ ($P < 0.10$).

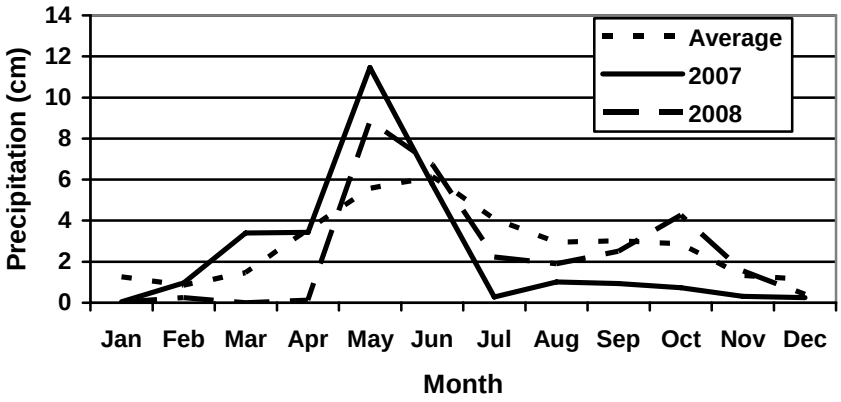

Figure 1. Average annual precipitation (30-yr), 2007, and 2008 precipitation by month for Miles City, MT (NOAA, 2007, 2008).

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THE EFFECTS OF REDUCING DIETARY PHOSPHORUS BY THE ADDITION OF BLUEGRASS STRAW TO THE RATIONS OF EARLY TO MID-LACTATION HOLSTEIN DAIRY COWS

A.C. Huisman, S.M. Cobb, R. L. Kincaid, J. J. Michal, and K. A. Johnson
Washington State University, Pullman 99164

ABSTRACT: Phosphorus (P) excretion by dairy cows is a growing environmental concern. To examine the impact of partially substituting bluegrass straw for alfalfa hay to reduce dietary P in early to mid-lactation (114 DIM), 12 dairy cows were used in a switchback design. Cows were fed a control TMR (C) or a TMR in which 10% of alfalfa hay DM was replaced by bluegrass straw (BGS) for 3 wk, diets were switched and cows fed for another 3 wk. Daily feed intake and orts were measured, milk and blood samples were collected on d 2, 16, and 37 and analyzed for milk composition and blood NEFA, urea nitrogen and P. In vitro digestibility of the diets and forage samples was determined using an Ankom Daisy Incubator. Inclusion of BGS in the diet reduced the concentration of CP and P (0.39% vs. 0.33%) but increased ADF and NDF. Average DMI (26.4 kg), total in vitro digestibility, DM disappearance, feed costs, income over feed cost, milk fat, milk protein, milk lactose, blood NEFA, blood urea N, and blood P were unaffected by the addition of BGS. Milk yield was reduced by the BGS ($P<0.05$), averaging 37 kg for C and 35 kg for BGS. Fat corrected milk (FCM) was not different (36 kg and 38 kg, respectively). Milk solids not fat was decreased in BGS ($P<0.05$). Income from milk was reduced by inclusion of BGS ($P < 0.05$; \$15.56/cow/d for C vs. \$14.62/cow/d for BGS). Partial substitution of BGS for alfalfa hay in diets of early to mid lactation cows reduced the %P in the diet thereby reducing P intake. Although DMI and feed costs were not affected by adding BGS to the diet, milk yield was reduced. Partial replacement of alfalfa hay with BGS may help reduce fecal P to aide in P management.

Keywords: dairy, phosphorus, bluegrass straw

Introduction

To ensure low P intakes do not reduce milk yield, cows are often fed 20 to 25% more P than current NRC recommendations (NRC, 2001; Toor et al., 2005). In addition, many commonly used byproduct feeds have high P concentrations. Excess P is excreted in the feces, causing accumulation of P in soils which contributes to eutrophication in waters (Toor et al., 2005). Therefore, it is important to meet but not exceed P requirements of dairy cows so that P excretion is reduced.

O'Rourke et al. (2007) fed late lactation Holstein cows a diet in which dietary P was reduced because bluegrass straw (BGS) partially replaced alfalfa hay. The BGS reduced P intake and fecal P without affecting milk yield. The objective of the current work was to determine if BGS could partially replace alfalfa hay in diets of early to mid-lactation dairy cows without affecting milk yield or composition.

Materials and Methods

Two groups of Holstein cows were housed in freestall barns with headlock gates in the feed alleyways. Two subsets of 12 cows in early to mid lactation (114 DIM) were selected from within the two larger groups for data collection. Selected cows were between 60 and 200 DIM and paired based on milk yield. All

cows within a group were randomly assigned either a control TMR or a TMR containing 10% BGS (DM). Diets are described in Tables 1 and 2. Cows were fed once per day at 0800 h and milked daily at 0900 and 2100 h. Cows within each pen were fed their respective diet for 3 wk. The diets were switched between pens and cows fed for an additional 3 wk in a switch back design. Daily feed intake data were collected for the entire group but fecal, milk and blood data were collected for the subset of 24 cows.

Fresh TMR was sampled daily by collecting 150g at 1 m intervals. Orts also were sampled. Particle separation was performed on both fresh TMR and orts using a Penn State Particle Separator to determine sorting (Heinrichs and Kononoff, 2002). Fecal samples were collected using rectal grab sampling on d 2, 16 and 37. Between 100 and 200g of feces were collected from each cow in a disposable OB glove and taken to the lab for analysis.

Feed and fecal samples were dried in a 60°C oven for 48 h to determine DM (O'Rourke et al. 2007). Once dry, samples were ground through a 2-mm screen in a Wiley mill (Arthur H. Thomas and Co., Philadelphia, PA). Total DM was determined by drying a sub-sample of the ground sample in a 100°C oven for 24 h. Feed and fecal samples were analyzed in duplicate for ash, CP, NDF, ADF, and P (AOAC, 2001). NDF and ADF were determined using an Ankom Fiber Analyzer. Total in vitro digestibility was determined using an Ankom Daisy Incubator. A composite sample of each diet was obtained by combining a 100g subsample of each ground weekly feed sample. Composite samples were divided into fiber bags and placed in glass jars containing 1600mL of buffer solution (pH 6.8) pre-warmed to 39°C. Rumen inoculum was obtained from cannulated cows fed a 100% BGS diet. Rumen inoculum (400mL) was filtered through cheesecloth into each jar that was placed in the Daisy Incubator, continually rotated and kept at 39°C. After 48 h jars were removed and cooled at 4°C for 24 h. Bags were rinsed in tap water, dried in a 100°C oven and weighed to determine total IVTD.

Blood was collected from the coccygeal vein of all 24 cows on d 2, 16 and 37 and allowed to clot in the collection tube. Blood was centrifuged at 2000 x g for 20 min to obtain serum. Serum was transferred to microcentrifuge tubes and stored in a freezer at -20°C. Serum P concentration was determined using colorimetric assay (AOAC, 2001). BUN was determined using BioAssay Systems QuantiChrom Urea assay kit (DIUR-500). Serum NEFA was determined using a Wako HR series NEFA-HR(2) kit.

Milk was collected on d 2, 9, 16, 23, 30 and 37. Samples from both AM and PM milking were combined. A subsample was preserved with bronopol and sent to DHIA for analysis. A second subsample was untreated and frozen until analysis for milk urea nitrogen (MUN). Milk was deproteinated with 10% TCA prior to MUN analysis which was done using the BioAssay Systems QuantiChrom Urea assay kit (DIUR-500). Fat corrected milk (FCM) was calculated using the following equation: FCM = 0.432 * milk yield (kg) + 16.32 * milk fat (kg) (Brog, 1971).

Statistical analysis was performed using PROC GLM of SAS (v. 9.1; SAS Institute Inc., Cary, NC). The data were analyzed for the effects of treatment and period. Serum measures, fecal data, and milk data from cows were analyzed using initial values as a covariate to eliminate cow effect. Feed intake, feed costs, income and income over feed cost data were analyzed for the effects of treatment and period. Feed sorting data were analyzed for effect of treatment and screen size.

Results and Discussion

Inclusion of BGS reduced dietary CP and P concentrations while increasing NDF and ADF (Table 2). Total in vitro digestibility was unaffected by treatment. DMI also was unaffected by treatment (Table 3). Intake of P was reduced by 17.5 g/d and N intake was reduced 162 g/d by inclusion of BGS.

Particle size distribution was affected by treatment (Table 4). Inclusion of BGS increased the long particle (>19.0 mm) fraction of the diet. Both control and BGS diets had more long particles than recommended (Heinrichs and Kononoff, 2002). Sorting of the diet occurred in both diets. Orts from BGS the diet had a higher percentage of long particles than control, indicating greater sorting in cows fed BGS. When the length of the forage is longer, cows increasingly discriminate against the long

particles in the diet (Leonardi and Armentano, 2003).

Milk yield was reduced in cows fed BGS (37.74 vs. 35.48 kg/d) but fat corrected milk was not affected (38.54 vs. 36.13 kg/d). Milk solids-not-fat was reduced (8.8 vs. 8.72%), whereas milk fat, milk protein, lactose and somatic cell count were unaffected by treatment. Income from milk was reduced, but income over feed cost (IOFC) was not affected (Table 3). The reduction in income with no reduction is IOFC is likely due to the (non-significant) reduction in feed costs for the BGS diet (Table 3).

Serum concentrations of NEFA, BUN and P were not affected by BGS in the diet. However, BUN values were high (20.79 mg/dL) compared to target BUN values between 13 and 17 mg/dL for cows producing 36 kg of milk per day (Jonker, 1999), indicating an inefficient N use by all cows (Roseler et al. 1993). MUN values were correlated with BUN (r^2 = 0.74). There was no effect of treatment on MUN during the first 3 wk, however, during wk 4 and 5 MUN values were lowered in cows fed BGS. This delayed effect could be due to urea recycling via saliva into the rumen, buffering short-term changes in N intake (Firkins et al. 2007).

Fecal P was reduced by inclusion of BGS in period 1 (0.73 vs. 0.61%), but not in period 2 (0.65 vs. 0.79%). Previous research reported a reduction in fecal P by including BGS in the diets of late lactation cows (O'Rourke, 2007), and although dietary P intake was reduced, fecal P was not reduced in the present trial.

Implications

Inclusion of BGS in the rations of early to mid-lactation dairy cows reduced N and P intakes. Dry matter intake, serum measures, and milk composition were unaffected by the BGS. However, milk yield and income from milk were reduced but not IOFC. The high BUN and MUN values reflect the high dietary CP level and possibly indicate need for more fermentable carbohydrate in the ration. Although fecal P % was not affected, dietary P was reduced by inclusion of BGS, giving producers an option for controlling P content of the ration.

Acknowledgements

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Tables

Table 1: Ingredient composition of control and bluegrass straw diets (DM).

Ingredient	Control Diet, %	BGS Diet, %
Alfalfa haylage	27.3	27.3
Alfalfa hay	25.4	25.4
Bluegrass straw	0	10
Whole cotton seed	6.36	6.36
Concentrate mix	36.4	36.4
DDGS[a]	4.5	4.5
Total	100	100

[a] DDGS = Distiller's dried grains and solubles

Table 2: Nutrient analysis of control and bluegrass straw diets (DM).

Nutrient, %	Control Diet	BGS Diet
DM	58.1	58.2
CP	21.5	18.2
P	0.39	0.33
ADF	24.5	26.8
NDF	32.4	38.3
IVTD	81.23	79.34

Table 3: Effect of including bluegrass straw in diets on daily DMI, P intake, feed costs, income, FCM and IOFC.

Data	Control	BGS	S.E
DMI, kg/cow/d	26.6	26.2	1.5
P intake, g/cow/d	104	86.5	
Feed costs, $/cow/d	8.1	7.8	0.6
Milk income, $/cow/d	15.6[a]	14.6[b]	0.2
3.5 FCM, kg/cow/d	38.5	36.1	0.6
IOFC[1], $/cow/d	1.95	1.88	0.04

[a,b] Means in the same row with different superscripts differ ($p<0.05$)
[1] IOFC = income over feed cost

Table 4: Particle size distribution of fresh TMR and orts in control and bluegrass straw diets.

Screen Size, mm	Control		BGS	
	Fresh TMR, %	Orts, %	Fresh TMR, %	Orts, %
> 19.0	17.24[a]	43.75[b]	27.06[c]	53.85[d]
19.0-8.0	30.00[a]	23.14[b]	24.64[c]	17.57[d]
8.0-1.18	36.71[a]	18.49[b]	33.25[c]	14.62[d]
< 1.18	13.97[a]	6.64[b]	13.52[c]	5.38[d]

[a,b,c,d] Means in the same row with different superscripts differ ($p<0.05$)

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EFFECTS OF MATERNAL NUTRITIONAL PLANE AND SELENIUM SUPPLY ON CELLULARITY ESTIMATES OF NEONATAL LAMB JEJUNAL MUCOSA, HEART, AND SKELETAL MUSCLE

T. L. Neville*†, A. M. Meyer†, J. J. Reed†, J. B. Taylor‡, L. P. Reynolds†, D. A. Redmer†, K. R. Maddock Carlin†, K. A. Vonnahme†, and J. S. Caton†

†Center for Nutrition and Pregnancy, Department of Animal Sciences, North Dakota State University, Fargo 58108

‡USDA-ARS, U.S. Sheep Experiment Station, Dubois, ID 83423

ABSTRACT: Objectives were to investigate the effects of maternal nutrition and Se supply during gestation on lamb jejunal mucosa, heart, and skeletal muscle RNA, DNA, and protein. Rambouillet ewe lambs (n = 84) were allotted to a 2 x 3 factorial design including dietary factors of Se [adequate Se (**ASe**; 11.5 µg/kg BW) or high Se (**HSe**; 77.0 µg/kg BW)] and nutritional plane [60% (**RES**), 100% (**CON**), or 140% (**HIGH**)]. At breeding Se treatments were initiated followed by nutritional treatments on d 40 of gestation. At birth, lambs (n = 13, 14, 14, 12, 13, and 15 for ASe-RES, ASe-CON, ASe-HIGH, HSe-RES, HSe-CON, and HSe-HIGH, respectively) were removed from ewes before nursing, placed in a common pen, and group fed until necropsy at 20.6 ± 0.9 d of age. Maternal nutritional plane affected ($P \leq 0.07$) offspring jejunal mucosal scrape concentration (mg/g) and total content (mg) of DNA where RES was least, HIGH greatest, and CON intermediate. Plane of nutrition also affected ($P = 0.07$) right ventricle DNA content where RES (189.8 ± 11.8 mg) was least, HIGH (208.2 ± 11.2 mg) intermediate, and CON (227.7 ± 11.3 mg) greatest. Maternal Se supplementation decreased ($P = 0.08$) left ventricle protein:DNA in offspring. For lamb right ventricle, RNA concentration was greatest ($P = 0.05$) for ASe-RES and least for HSe-RES with all other treatments intermediate. However when lamb right ventricle RNA was expressed as total content, HSe-RES was least ($P = 0.02$), ASe-HIGH intermediate, and all other treatments were greater. When RNA:DNA was calculated in right ventricle, ASe-RES and HSe-HIGH were greatest ($P = 0.02$), ASe-CON intermediate, and ASe-HIGH, HSe-RES, and HSe-CON least. Skeletal muscle RNA concentration and RNA:DNA were least ($P < 0.05$) for ASe-HIGH, intermediate for HSe-RES and HSe-CON, and greatest for ASe-RES, ASe-CON, and HSe-HIGH. These data indicate cellularity estimates have tissue specific responses to maternal nutritional plane and Se supply.

KEYWORDS: cellularity, maternal nutrition, neonatal lambs, selenium

Introduction

Selenium, an essential trace mineral, is important for normal growth and development (Sunde, 1997). Selenium is regulated by the FDA to an inclusion limit less than 0.3 ppm (FDA 21CFR573.920), however many plants grown on rangelands in North and South Dakota contain much higher levels of Se due to the geographic formations in these areas (Rosenfeld and Beath, 1964). Global nutrient restriction or overfeeding during adolescence in ewe lambs alters normal growth and development of the fetus and placenta (Wallace et al., 2000; Reed et al., 2007). The lifelong regulation of normal growth, development, and nutrient utilization are likely programmed *in utero* (Wu et al., 2006).

Selenium supplementation and nutrient restriction have had varying results on cellularity estimates in near term fetuses in several studies (Reddy et al., 2006; Reed et al., 2007; Neville et al., 2008). Chemical form of Se supplement (selenate vs. high Se wheat) and level (3 vs. 15 ppm) affected small intestine RNA:DNA and protein:DNA in fetuses at d 134 of gestation (Neville et al., 2008). Fetal skeletal muscle and heart tissue had decreased protein:DNA due to maternal dietary restriction in fetuses at d 135 of gestation (Reed et al., 2007). Maternal nutrient restriction from d 50 to 90 of gestation increased RNA:DNA in the small intestine of fetuses at d 135 of gestation (Reddy et al., 2006). Higher jejunal mucosal DNA was reported in lambs (180 d of age) born to ewes that were either non-Se supplemented control fed or Se supplemented nutrient restricted (unpublished, Caton). The effects of nutrient restriction or overfeeding in conjunction with Se supplementation on neonatal lambs reared independent of their dams have not been studied; therefore, the objective of this study was to investigate the influence of maternal plane of nutrition and Se supplementation on cellularity estimates in jejunal mucosa, heart, and skeletal muscle from 20 d old lambs.

Materials and Methods

Animals and Diets. This experiment was approved by the Institutional Animal Care and Use Committee at North Dakota State University. Eighty-four pregnant Rambouillet ewe lambs (52.1 ± 6.2 kg; d 40 ± 3 d of gestation) were individually housed in 0.91 × 1.2 m pens. Ewes were randomly allotted to 1 of 6 treatments in a 2 x 3 factorial array. Main effects evaluated were dietary levels of Se [initiated at breeding; adequate (**ASe**; 11.5 µg Se•kg BW^{-1}•d^{-1}) vs. high (**HSe**; 77.0 µg Se•kg BW^{-1}•d^{-1})], and nutritional plane [initiated at d 40 of gestation; 60% (**RES**), 100% (**CON**), and 140% (**HIGH**) of requirements for gestating ewe lambs].

All diets were fed once daily in a complete pelleted form (0.48 cm diameter; based on wheat middlings, beet pulp, alfalfa meal, and ground corn). Three pellet formulations (basal, high Se, and concentrated Se pellets) were blended to meet Se and ME intake based upon the Se treatment and nutritional plane of each ewe. The basal pellet contained 15.9% CP and 2.81 Mcal/kg ME DM basis. Selenium sources used were Se-enriched wheat mill run to

replace wheat middlings in basal diet to make a high Se pellet (16.6% CP and 2.82 Mcal/kg ME DM basis) and purified seleno-methionine added to achieve 37.1 ppm Se in the concentrated Se pellet (16.2% CP and 3.01 Mcal/kg ME DM basis). Nutrient requirements were based on NRC (1985) recommendations for 60 kg pregnant ewe lambs during mid to late gestation (weighted ADG of 140 g). Body weight was measured every 14 d and diets were adjusted accordingly.

Parturition to Necropsy. Upon parturition, lambs were immediately removed from dams before nursing occurred. Lambs were fed artificial colostrum (Acquire, APC, Inc., Ankeny, IA) by bottle within 30 min of birth. Colostrum was fed in six feedings over the first 20 h after birth (providing 10.64 g IgG/kg BW). From 24 h until harvest, lambs were fed milk replacer (Super Lamb Instant Milk Replacer, Merrick's Inc., Middleton, WI) and had full access to fresh creep feed and water. At harvest (20.6 ± 0.9 d of age), lambs were stunned by captive bolt (Supercash Mark 2, Acceles and Shelvoke Ltd., England), exsanguinated, and necropsied. Small intestine and heart weights were recorded. A 10-cm sample of small intestine was removed, gently washed in PBS buffer, weighed, placed on a polyethylene cutting board, and opened luminal side up. Mucosal tissue was separated (scraped) from the remaining tissue with a glass histological slide. Remaining tissue was re-weighed to calculate the percentage of mucosa. Five 1-g samples of the mucosa, left and right heart ventricles, and skeletal muscle (loin) were snap frozen in super-cooled isopentane (submerged in liquid nitrogen), and stored at -80°C until analysis.

Cellularity Estimates. Tissue homogenates were analyzed for concentrations of DNA and RNA using the diphenylamine (Johnson et al., 1997) and orcinol procedures (Reynolds et al., 1990). Protein in tissue homogenates was determined with Coomassie brilliant blue G (Bradford, 1976), with bovine serum albumin (Fraction V; Sigma, St. Louis, MO) as the standard (Johnson et al., 1997). Concentration of DNA was used as an index of hyperplasia, and RNA:DNA and protein:DNA ratios were used as an index of hypertrophy (Swanson et al., 2000; Scheaffer et al., 2003; Soto-Navarro et al., 2004). Tissue DNA, RNA, and protein contents were calculated by multiplying DNA, RNA, and protein concentration by fresh tissue weights (Swanson et al., 2000; Scheaffer et al., 2003; Scheaffer et al., 2004). Fresh heart weight was utilized for the left and right ventricles content calculations and weight of mucosal scrape was calculated by multiplying percentage mucosa by small intestinal weight.

Statistics. Data were analyzed as a completely randomized design with a 2 × 3 factorial arrangement of treatments using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The model contained effects for Se (ASe vs. HSe), nutritional plane (RES, CON, and HIGH), and Se × nutritional plane interaction. Only singleton lambs were utilized in the analysis resulting in 13, 14, 12, 10, 13, and 13 lambs for ASe-RES, ASe-CON, ASe-HIGH, HSe-RES, HSe-CON, and HSe-HIGH, respectively. Sex was included in the model and retained when significant ($P < 0.15$). When main effects or interactions were present ($P < 0.10$), means were separated by least significant difference. Main effects were considered significant when $P < 0.10$.

Results

When interactions were present they will be discussed; however when absent, main effect differences will be presented. Maternal nutritional plane in conjunction with Se supplementation resulted in skeletal muscle having the greatest ($P \leq 0.04$; Table 1) RNA concentration (mg/g) and RNA:DNA in lambs born to ewes from ASe-RES, ASe-CON, or HSe-HIGH treatments and the least RNA concentration and RNA:DNA in lambs from ASe-HIGH ewes. No differences ($P \geq 0.42$) in skeletal muscle DNA, protein, or protein:DNA were found.

Mucosal scrape from lambs born to HIGH fed ewes had the greatest ($P \leq 0.07$) DNA concentration and content with CON offspring intermediate and RES least (5.17, 5.30, and 5.71 ± 0.18 mg/g and 1382, 1556, and 1719 ± 93 mg for DNA concentration and content in RES, CON, and HIGH, respectively). No differences ($P \geq 0.11$) were found in RNA, protein, RNA:DNA, or protein:DNA.

For heart tissue, right and left ventricles were analyzed separately. In the left ventricle, Se supplementation decreased ($P = 0.08$) protein:DNA (35.7 and 29.3 ± 2.6 for ASe and HSe, respectively). In the right ventricle, lambs born to CON ewes had greatest ($P = 0.07$) DNA content with HIGH intermediate and RES the least (189.8, 227.7, and 208.2 ± 11.8 mg for RES, CON, and HIGH, respectively). Right ventricle RNA concentration was least ($P = 0.05$) in HSe-RES offspring and greatest in ASe-RES offspring with all other treatments intermediate. When right ventricle RNA was expressed as total content HSe-RES was least ($P = 0.02$) and ASe-HIGH intermediate compared to all other treatments. The calculation of right ventricle RNA:DNA indicated the greatest ($P = 0.02$) ratio was from ASe-RES and HSe-HIGH offspring with ASe-CON intermediate and all other treatments least.

Discussion

The increase in RNA:DNA ratio in skeletal muscle of ASe-RES, ASe-CON, and HSe-HIGH is an indicator of increased hypertrophy, an increase in the size of cells. This result is dissimilar to previous results where at d 135 of gestation, fetuses had decreased hypertrophy due to maternal nutrient restriction (Reed et al., 2007) and at 180 d of age lambs had no differences in skeletal muscle hypertrophy (unpublished, Maddock-Carlin). Perhaps these differences are due to stage of growth or age relative to the maternal nutritional insults during gestation.

Our current study demonstrated increased hyperplasia in mucosal scrape of offspring from HIGH fed ewes. Jejunal mucosal scrape DNA concentration was altered due to maternal nutritional plane and Se supplementation in lambs harvested at 180 d of age where lambs from ASe-CON and HSe-RES had increased DNA concentrations compared to HSe-CON and HSe-HIGH (unpublished, Caton). Hyperplasia may result in the gross enlargement of an organ; however no differences were reported in small intestinal weight in the current study (Meyer et al., 2009). Because weight of the small intestine at d 20 is similar among treatments, the increased hyperplasia may be

indicative of larger villi that would provide increased surface area for absorption of nutrients. This could alter the health status and performance of the animal as it grows and into adult life.

In the right ventricle, increased RNA:DNA indicates increased hypertrophy in ASe-RES and HSe-HIGH. In previous research, decreased protein:DNA was reported due to ewe dietary restriction during gestation (Reed et al., 2007). Ventricular hypertrophy can be associated with diseased states. The stress resulting from maternal nutrient restriction or overfeeding along with level of Se supplementation on the lamb *in utero* may be the factors driving increased right ventricular hypertrophy. Other researchers (Vonnahme et al., 2003) have demonstrated bilateral ventricular hypertrophy due to maternal nutrient restriction and they hypothesized this to be the result of placental vascular resistance. Additionally, Long et al. (2009) reported increased heart and right ventricle weights per unit of BW in d 125 bovine fetuses due to intrauterine growth restriction from nutrient restriction. These alterations in hypertrophy may have long term consequences on heart health (Godfrey and Barker, 2000; Wu et al., 2006). In the left ventricle, maternal Se supplementation decreased cell size, perhaps moderating cell growth.

In summary, tissue specific differences in hypertrophy and hyperplasia were observed. Impacts of these changes, which resulted from maternal nutritional plane and/or Se supplementation, on life long performance remain to be determined.

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Table 1. Interactive means of maternal selenium supply and nutritional plane on cellularity estimates in skeletal muscle, mucosal scrape, and right and left heart ventricles of lambs at 20 d of age

	Treatments[1]							*P*-values[2]		
Item	ASe-RES	ASe-CON	ASe-HIGH	HSe-RES	HSe-CON	HSe-HIGH	SE	Nut	Se	Nut*Se
Loin										
RNA, mg/g	2.08[a]	2.00[a]	1.63[b]	1.82[ab]	1.91[ab]	1.99[a]	0.14	0.42	0.98	0.04
DNA, mg/g	1.32	1.28	1.33	1.33	1.28	1.26	0.07	0.76	0.68	0.81
Protein, mg/g	82.8	79.5	89.9	76.0	83.1	81.9	6.7	0.55	0.46	0.56
RNA:DNA	1.59[a]	1.58[a]	1.28[b]	1.38[bc]	1.49[ac]	1.59[a]	0.09	0.44	0.97	0.007
Protein:DNA	65.3	63.7	70.7	58.9	66.0	66.8	6.5	0.54	0.58	0.74
Mucosal Scrape										
RNA, mg/g	5.19	5.35	6.10	5.46	5.65	5.25	0.34	0.54	0.71	0.11
RNA, mg	1365	1529	1910	1513	1775	1561	177	0.18	0.91	0.14
DNA, mg/g[3]	4.91	5.36	5.94	5.42	5.25	5.48	0.27	0.07	0.93	0.15
DNA, mg[3]	1281	1494	1815	1484	1617	1621	140	0.04	0.67	0.26
Protein, mg/g	27.1	28.6	29.2	27.1	27.0	27.7	3.1	0.90	0.65	0.95
Protein, mg	7335	8379	9344	7704	8468	8317	1337	0.56	0.85	0.83
RNA:DNA	1.06	1.01	1.03	1.02	1.11	0.97	0.05	0.29	0.90	0.12
Protein:DNA	5.62	5.47	5.00	5.01	5.32	5.12	0.59	0.80	0.62	0.80
Right Ventricle										
RNA, mg/g	2.22[a]	2.08[ab]	1.80[bc]	1.69[b]	1.98[ab]	2.13[ac]	0.19	0.89	0.48	0.05
RNA, mg	146.5[a]	136.0[a]	124.4[ab]	103.5[b]	150.7[a]	154.8[a]	14.7	0.36	0.95	0.02
DNA, mg/g	2.99	3.21	3.10	3.00	3.25	2.77	0.20	0.21	0.53	0.53
DNA, mg[3]	194.6	211.7	213.7	185.0	243.8	202.7	16.2	0.07	0.77	0.30
Protein, mg/g	31.4	29.4	27.7	30.6	34.9	25.1	3.8	0.21	0.80	0.45
Protein, mg	2089	1901	1930	1884	2659	1821	308	0.31	0.51	0.16
RNA:DNA	0.73[a]	0.67[ab]	0.60[b]	0.57[b]	0.64[b]	0.78[a]	0.06	0.78	0.92	0.02
Protein:DNA	10.52	9.40	9.08	10.31	11.62	8.81	1.32	0.34	0.55	0.48
Left Ventricle										
RNA, mg/g	2.22	2.11	2.11	2.38	2.13	2.23	0.16	0.43	0.37	0.87
RNA, mg	144.7	142.4	147.7	145.0	160.0	161.5	14.9	0.77	0.34	0.80
DNA, mg/g	3.40	3.16	3.54	3.91	3.33	3.77	0.36	0.32	0.25	0.86
DNA, mg	216.6	216.4	243.4	235.0	252.2	267.6	26.3	0.44	0.18	0.93
Protein, mg/g	115.3	93.3	111.0	97.4	93.0	103.0	11.1	0.29	0.28	0.68
Protein, mg	7555	6291	7740	6038	7348	7711	912	0.44	0.81	0.30
RNA:DNA	0.70	0.73	0.65	0.65	0.69	0.65	0.07	0.64	0.57	0.92
Protein:DNA[3]	38.4	33.3	35.4	27.6	29.4	30.8	4.9	0.90	0.08	0.71

[1]ASe-RES = offspring from ewes fed 11.5 ug/kg BW Se (ASe: no added Se), restricted to 60% of control;
ASe-CON = offspring from ewes fed 11.5 ug/kg BW Se, consuming requirement level of energy (control);
ASe-HIGH = offspring from ewes fed 11.5 ug/kg BW Se, fed to 140% of control;
HSe-RES = offspring from ewes fed 77 ug/kg BW Se (HSe; high Se) , restricted to 60% of control;
HSe-CON = offspring from ewes fed 77 ug/kg BW Se, consuming requirement level of energy (control);
HSe-HIGH = offspring from ewes fed 77 ug/kg BW Se, fed to 140% of control.
[2]Probablity values for effects of Se supply (Se), nutritional plane (Nut), and the interaction.
[3]Main effect means presented in text.
[abc]Means differ by $P < 0.10$.

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IMPACT OF GASTROINTESTINAL PARASITES ON ANTIBODY TITER RESPONSES TO VACCINATION AND IBR CHALLENGE

J.S. Schutz[1], E.D. Sharman[1], N.E.Davis[1], T. Shelton[2], S. Nordstrom[2], J. Hutcheson[2], and T.E. Engle[1,]
[1]Department of Animal Science, Colorado State University, Fort Collins, CO 80523, [2]Intervet-Schering Plough Animal Health, Desoto, KS 66018

ABSTRACT: Thirty-three colostrum deprived Holstein bull calves (initial BW of 131.3 kg ± 4.2) were utilized to determine the impact of timing of anthelmintic administration relative to vaccination on antibody titer response to vaccine components, subsequent rectal temperature and antibody response to an IBR challenge. When calves were at least three mo of age they were randomly sorted into individual pens and placed into one of three treatment groups (n = 11) 1) dewormed 2 wk prior to vaccination (**DPV**); 2) dewormed at the time of vaccination (**DV**); and 3) Control: not dewormed (**CONT**). All calves were inoculated with infective larvae of brown stomach worms (*Ostertagia ostertagi*) and intestinal worms (*Cooperia* spp.) on d 1, 7, 10, 14, and 18 for a total dose of 235,710 infective larvae per calf. Calves (DPV and DV) were dewormed with a 10% fenbendazole suspension at 5 mg/kg BW two wk prior to vaccination. On d 35, all treatments were vaccinated and DV calves were dewormed. Weekly fecal egg counts, blood, and rectal temperatures were collected throughout the experiment. Statistical analyses of data were preformed for a completely randomized block design utilizing the Mixed procedure of SAS (2003). Where appropriate, repeated measures analysis were utilized. All groups had elevated titers for IBR, BVDV 1, BVDV 2, and PI-3 by d 15 post vaccination. Animals dewormed at the time of vaccination had higher ($P < 0.05$) titers to BVD 1. On d 88 all calves were challenged with IBR (4 ml of 1.8×10^7 CCID) and blood samples were obtained on d 0, 1, 3, 4, 6, 8, 10, and 12 post inoculation. Post IBR inoculation all groups had elevated ($P < 0.05$) rectal temperatures. The CONT group had greater ($P < 0.05$) rectal temperatures than DPV and DV calves on d 88, 89, and 97. All groups had elevated titers for IBR, BVD, and BVD 2 during the IBR challenge period. It can be concluded that deworming prior to or at vaccination reduced parasite burden and decreased rectal temperature elevation following an IBR challenge

Key Words: gastrointestinal parasite, immunity, calves, vaccination

Introduction

Gastrointestinal parasite burden is one of the largest health concerns for ruminants worldwide (Armour, 1980). Animal performance has been shown to decline in relationship to parasite burden (Lee, 1955: Cox and Todd, 1962: Anderson et al., 1965; Ward et al., 1991: Reinhardt et al., 2006). Gastrointestinal parasitism results vary widely in the host animal; from subclinical effects to death depending upon parasite load, animal age, plane of nutrition, and overall health status (Hawkins, 1993). Economic data indicates that calves treated with anthelmintics resulted in an improvement of $0.0754 to $0.1409 / kg gain after 41 days post treatment (Leland et al., 1980). Additionally, Grimson et al. (1987) reported that average sale prices were greater for calves given antiparasitic treatments vs. untreated calves.

Vaccinations are arguably, the most cost effective means for preventing disease, especially in feedlot environments. However, it is critical that good management practices are implemented in conjunction with vaccination programs to ensure that vaccine efficacy is not compromised. Most calves are vaccinated for respiratory type infections and dewormed at weaning which can be directly prior to the calves entering an on site background facility or being transported to a feedlot (Bagley, 2001). Several reports have indicated that cytokine patterns associated with the immune system responding to parasite infestation can interfere with the response to non-parasitic antigens which may affect the efficiency of a vaccination (Urban, 2007). Because of the correlation between time of deworming and vaccination, it is critical to determine the impact of deworming calves prior to arriving at a feedlot. Therefore, the objective of this experiment was to determine the impact of timing of anthelmintic administration relative to vaccination on antibody titer response to vaccine components and subsequent rectal temperature and antibody response to an IBR challenge.

Materials and Methods

Prior to the initiation of this experiment, care, handling, and sampling of the animals defined herein were approved by the Colorado State University Animal Care and Use Committee.

Thirty-three colostrum deprived Holstein bull calves (BW 131 kg ± 4.24) were utilized in this experiment. Calves were obtained from a single local dairy immediately after birth and transported to Colorado State University's Agricultural Research Development and Education Center (ARDEC) located in Fort Collins, CO. Upon arrival, all calves were weighed, given a unique numerical identification ear tag, injected (i.m.) with 1 ml of a vitamin A and D solution (AgriPharm, Memphis, TN) and rectal temperatures were obtained. Calves were then housed in individual calf huts (1.5 x 1.9 m).

Growing phase: At two weeks of age, a jugular blood sample was collected in a non-heparinized vacutainer tube (Becton Dickenson Co., Franklin Lakes, NJ) for the determination of Infectious Bovine Rhinotracheitis (**IBR**), Bovine Viral Diarrhea Type 1 (**BVDV I**), Bovine Viral Diarrhea Type 2 (**BVDV II**), and Parainfluenze-3 (**PI-3**)

antibody titers. Calves were bottle-fed whole milk twice daily for the first two weeks of life. Calves were then gradually transitioned to a milk replacer (MAXI CARE 22-20 NT Medicated dairy herd & beef calf milk replacer) diet over a two week period (Step 1: 60% whole milk and 40 % milk replacer; step 2: 75% milk replacer and 25 % whole milk; and step 3: 100% milk replacer). Calves remained on milk replacer for approximately 45 days. Calves had ad libitum access to water and medicated calf starter (20% crude protein, 7% crude fiber, and 2.25 % crude fat) 3 d post birth. Calves were weaned when they were consuming 1.81 kg of starter ration for five consecutive days (approximately 60 days of age).

Once weaned, calves were fed once daily in the morning and gradually transitioned to an alfalfa-steam flaked corn based growing diet (Table 1). Diets were formulated to meet or exceed all nutrient requirements for growing Holstein bull calves (NRC, 1989). Once transitioned to the growing diet, calves were fed twice daily at 0700 and 1600 h in amounts adequate to allow ad libitum access to feed throughout the day. Orts were recorded and weighed daily. When all calves were weaned and acclimated to the basal growing diet, calves were moved from the calf huts into individual pens (2 x 13m) equipped with automatic water fountains and a concrete feed bunk. All calves were fed the growing diet until the youngest calf was 3 mo of age.

Upon initiation of the experiment, individual body weights were obtained on two consecutive days and calves were blocked, by body weight and age, to one of three treatment groups. Treatments consisted of: 1) dewormed 2 weeks prior to vaccination (**DPV**); 2) dewormed at the time of vaccination (**DV**); and 3) Control: not dewormed (**CONT**). Individual feed intake and health status were recorded daily. Calves were determined to be morbid if rectal temperatures exceeded 39.7°C and were treated as prescribed by the attending veterinarian. Fecal samples, rectal temperatures, and a jugular blood sample (collected in a non-heparinized vacutainer tube; Becton Dickenson Co., Franklin Lakes, NJ) were obtained from each calf weekly.

Parasite inoculation phase: All calves (average age = 130 ± 22 d.) were inoculated with 23,571 infective larvae of brown stomach worms (*Ostertagia ostertagi*) and intestinal worms (*Cooperia* spp.) on d 1, 7, 10, 14, and 18 for a total dose of 235,710 infective larvae per calf. Immediately prior to and at each parasite inoculation a fecal grab sample was collected directly from the rectum. On d 18, one calf per treatment was euthanized and a complete necropsy was preformed. The rumen, a sample of rumen contents, abomasum, a sample of the omasum and reticulum, proximal jejunum, duodenum, bronchial and duodenum lymph nodes, peyers patches, kidney, heart, lungs, spleen, and liver were collected, weighed, and stored in a -20°C freezer for future histological analysis.

Deworming phase: On d 21 (3 weeks post initial parasite inoculation) treatment 1 (DPV) was dewormed with a 10% fenbendazole suspension (Safe-Guard®, Intervet, Millsboro, DE) at 5 mg/kg BW two weeks prior to vaccination. Subsequent to this, treatment 2 (DV) was dewormed at vaccination (d 35). All calves were vaccinated with 2 mL of a modified-live virus respiratory vaccine containing IBR, BVD type 1 and 2, PI-3, and BRSV (Vista® 5SQ, Intervet-Schering Plough Animal Health, Desoto, KS).

Post vaccination phase: Post vaccination, daily and weekly feed and health records and samples were obtained as previously outlined. On d 88 (53 days post vaccination) all calves were challenged intranasally with IBR (4 mL of 1.8 x 10^7 CCID) virulent BHV-1 Cooper strain suspension via nebulization (2 mL/nostril). Blood samples were obtained from all calves on d 0, 1, 3, 4, 6, 8, 10, and 12 d post inoculation and rectal temperatures were obtained every morning prior to feeding. Fourteen days post IBR challenge, all calves were euthanized and necropsies were performed (See figure 1 for the experimental timeline).

Analytical Procedures:

Blood preparation: Blood was stored on ice, transported to the laboratory and stored in a refrigerator at approximately 5°C for 12 hours to allow for clotting. Whole blood was then centrifuged at 1200 x *g* for 25 minutes at room temperature. The serum was harvested and stored in polyethylene tubes (12 mm X 75 mm) at -70°C. Every two weeks, serum was analyzed for IBR, BVDV I, BVDV II, and PI-3. Samples were diluted and equal amounts of IBR, BVD I, BVD II, and PI-3 were added to each samples dilution independently. The samples were then incubated and titer values determined.

Fecal egg counts: Approximately 100 g of fresh fecal matter was placed in an individual plastic bag, labeled, and placed on ice. The samples were refrigerated until analyzed. Samples were shipped to an independent laboratory (Animal Production Consulting, Lincoln, NE) for analysis. The Modified Wisconsin Sugar Flotation Technique (Cox and Todd, 1962) was utilized to examine each individual fecal sample. A 3 gm base sample was used for analysis. An egg per gram (**EPG**) count was determined by multiplying the total count by 150 and then dividing that number by 454.

Cytokine immunosorbent assay: Serum cytokine analysis was conducted utilizing an enzyme-linked immunosorbent assay. Briefly, the antibodies captured specific proteins in the sample which were added to each well. When unbound proteins were removed, a biotinylated detecting antibody was added and bound to a second site on the target protein. After this, excess detecting antibody was removed and streptavidin-horseradish peroxidase was added. This initiated a reaction with substrates to produce a signal. SuperSignal® ELISA Femto Chemiluninescent Substrate was used in this assay. The enzyme-substrate reaction produced a signal which was detected with a CCD camera. The amount of signal produced was directly proportional to the amount of each target protein in the sample (Thermo Fisher Scientific Pierce SearchLight Products, Woburn, MA).

Statistical analysis: Statistical analyses of data were preformed for a completely randomized block design utilizing the Mixed procedure of SAS (2003). Calf was considered the experimental unit. Where appropriate, repeated measures analysis were utilized. The model for ADG, DMI, EPG, and rectal temperature contained

treatment, day, and all possible interactions. Each period was analyzed independently. When treatment x day interactions were significant ($P < 0.05$) the effect of treatment was analyzed for each day. Logarithmic transformations were applied to all titer values.

Results and Discussion

Performance

Performance was similar across all treatments. This is in contrast to a study conducted by Horak et al. (1964) and Fox et al. (2002) where calves infected with *O. ostertagi* larvae resulted in reduced feed intake. Additionally, in an earlier study, ADG was shown to be greater in cattle treated with deworming agents (Flack et al., 1967). Moreover, Anderson et al. (1965), and Wiggin and Gibbs (1990), reported weight loss in cattle infected with internal parasites. However, it should be noted that the cited studies above raised cattle in groups whereas the current study raised calves confined in individual pens.

There were several time x period interactions for rectal temperature. During period one (parasite inoculation phase), CONT calves had higher ($P < 0.02$) rectal temperatures compared to DPV and DV treatments. Aitken et al. (1976) reported similar results as all heifers in their study revealed elevated rectal temperatures five days after inoculation. During period 3 (post vaccination phase), CONT calves had higher ($P < 0.04$) rectal temperatures compared to DPV and on d 49 DV calves had the highest rectal temperature throughout the period. During period 4 (IBR challenge phase), the CONT group had higher ($P < 0.01$) rectal temperatures on each sampling day except d 90 compared to the DPV and DV treatments.

There is a direct correlation between the fecal egg count and the amount of mature parasite within the host animal (Michel, 1967). By the beginning of period 2 (approximately 14 d after initial parasite inoculation) fecal eggs were detected. By design CONT animals had greater ($P < 0.0001$) fecal egg counts for periods 2 and 3. By period 4, the level of parasitism in the CONT group had decreased below the maximum egg counts for that group. This decrease in fecal egg count over time can be associated with the development of parasite resistance by the immune system of the host animal (Gordon, 1948).

Titers to vaccine components

All treatment groups had elevated titers for IBR, BVDV 1, BVDV 2, and PI-3 by d 15 post vaccination. Additionally, DV animals had higher ($P < 0.02$) titer concentrations for BVD during period 3 on d. 66, 73, 79. During period 1, the CONT calves had higher ($P < 0.02$) titer concentrations for PI-3. Although not significant, during periods 3 ($P< 0.12$) and 4 ($P<0.77$), CONT calves had a lower IBR concentration than DPV and DV groups.

Research in cattle investigating the impact of *Ostertagia ostertagi* infections indicate that *Ostertagia ostertagi* may have the ability to suppress both cell mediated and humoral immune responses (Hawkins, 1993). Therefore, animals with parasitic infections may have an altered cell-mediated immune response to non-parasitic antigens such as vaccines. This was reported in a study where mice were infected with *Schistosoma mansoni* and then given a non-parasitic antigen (Kullberg, 1992). Additionally, helminths infections can cause suppression of the host's immune response to vaccine components thus, possibly inhibiting the efficacy of the vaccine (Su et al., 2006).

Cytokine concentrations

Cytokine concentrations were similar across all treatments and no period interactions were detected. There was a tendency ($P < 0.09$) for CONT calves to have higher IL-4 concentrations. Additionally, there was a quadratic effect ($P < 0.03$) associated with TNF alpha concentrations.

Alterations in immune responses to foreign non-parasitic antigens have been observed in murine models infected with parasites. In a study conducted by Kullberg et al. (1991) the effect of a *Schistosoma mansoni* infection down regulated TH-1 cytokine response for IL-2 and IFN gamma as compared to immunized uninfected controls. A down regulated TH-dependant immune response could lead to an increased susceptibility to infection (Kullberg et al., 1991). *Fasciola hepatica* has been reported to cause an upregulation of TH-2 immune response, specifically associated with an increase in IL-4, which inadvertently inhibits certain TH-1 responses to a foreign antigen (Flynn et al., 2007). An inhibition of the TH-1immune response may decrease the ability of an animal to respond to an invading pathogen (Flynn et al., 2007).

Conclusions

These data indicate that deworming colostrum deprived Holstein bull calves two weeks prior to, or at the time of vaccination, reduced parasite burden and decreased rectal temperature elevation following an IBR challenge. Furthermore, timing of deworming relative to vaccination had no impact on titer or cytokine response to vaccine components or IBR challenge. Further studies are needed to gain a more fundamental understanding of the relationship between parasite burden and immunity.

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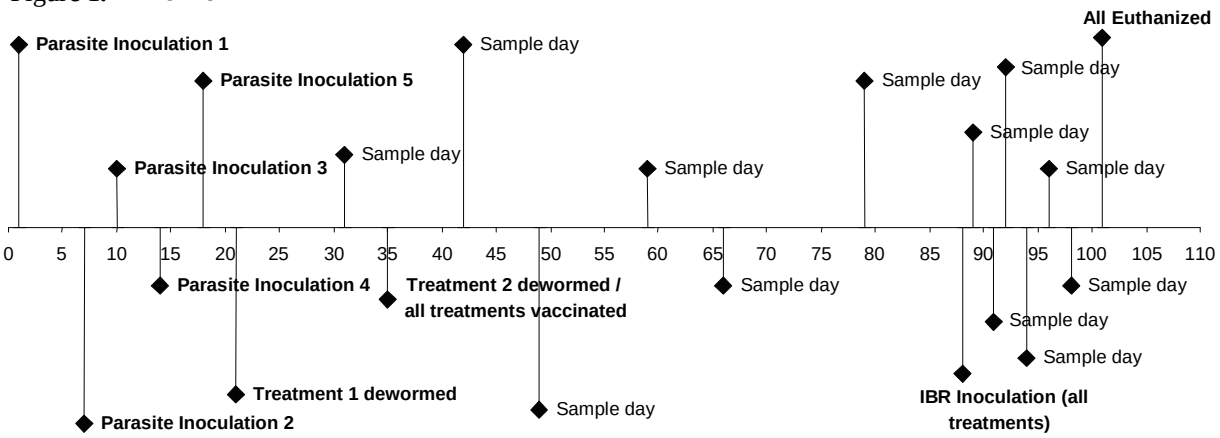
Table 1. Ingredient composition of basal diet.

Ingredient	%[a]
Alfalfa Hay	62.38
Steam Flaked Corn	15.60
Calf Concentrate[b]	18.02
Cane Molasses	4.00

[a] Dry matter basis

[b] Premix contained: crude protein = 32.10%, crude fat = 1.78, crude fiber = 7.76, dry matter = 91.15%

Figure 1. Timeline



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EVALUATION OF SACCHAROMYCES CEREVISIAE FERMENTATION PRODUCTS AS A NATURAL ALTERNATIVE TO AN IONOPHORE ON GROWTH PERFORMANCE, COST OF GAIN, AND CARCASS CHARACTERISTICS OF HEAVY-WEIGHT YEARLING BEEF STEERS

K. L. Swyers[1], J. J. Wagner[1], K. L. Dorton[2] and S. L. Archibeque[1]

[1]*Colorado State University, Fort Collins, CO 80523;* [2]*Diamond V Mills, Cedar Rapids, IA 52407*

ABSTRACT: Two-hundred fifty-two cross-bred yearling steers (BW = 406 ± 24 kg) were used in a completely randomized block design with a 2 x 2 factorial arrangement of treatments (7 pens / treatment) to evaluate the effects of dietary *Saccharomyces cerevisiae* fermentation product (**YC**) and monensin (**MON**) on growth performance, cost of gain (**COG**), and carcass characteristics. Dietary treatment factors were 1) with or without YC and 2) with or without MON in finishing diets with 19.7% inclusion of dried distiller's grains (**DDG**; DM basis). Both YC and MON were offered in the total mixed ration in place of an equal amount of cornmeal (DM basis; target intake = 2.8 g and 33 mg / kg of DMI, respectively). Steers were not implanted during the study. Body weights were collected on d 0, 28, 56, 84, 110, and 125. Initial and final BW was an average of two-day weights (d -1 and 0; d 124 and 125, respectively). Steers were shipped for harvest on d 125. There was no difference due to treatment on COG. However, overall ADG and BW were decreased ($P \leq 0.049$) in steers supplemented with YC. Twelfth rib fat thickness and frequency of USDA yield grade 4 and 5 carcasses tended to decrease when steers were fed YC alone, but tended to increase when steers were fed MON (YC x MON, $P = 0.072$ and $P = 0.086$, respectively). Feeding YC was associated with a lower ($P = 0.007$) HCW and a greater ($P = 0.003$) number of carcasses grading Choice. While not significant, YC steers had the highest percentage of yield grade 1 and 2 (81%) and premium Choice and Choice (67%) carcasses. These data indicate that feeding YC may improve carcass characteristics of steers finished at lower end weights, which could result in fewer days on feed. The effects of MON in the current study may have been limited in heavy yearling steers due to consumption of a finishing diet containing 19.7% DDG.

Key w ords: Beef, *Saccharomyces cerevisiae* Fermentation Product, Ionophore

INTRODUCTION

It is current conventional practice to administer antimicrobials, such as monensin (**MON**) as a growth-promoting feed additive in finishing beef feedlot diets in the U.S. Monensin, also referred to as an ionophore (Schelling, 1984), is widely used for improved feed efficiency, reduction in digestive upsets, and for the prevention of coccidiosis (Goodrich et al. 1984). However, there is growing consumer demand for natural and organically-grown beef (Thompson et al., 2007). The USDA (2009) designates that naturally-raised beef animals are to be grown without the use of growth hormones or antimicrobials. Therefore, establishing an economically competitive, natural alternative to ionophores, that fits under the USDA's designation for natural and organically-grown beef, and that does not compromise end-product quality, is of interest to researchers and beef producers. Such an alternative could be a *Saccharomyces cerevisiae* fermentation product (yeast culture; **YC**). *Saccharomyces cerevisiae* fermentation products have been shown to stimulate rumen bacterial yield by providing soluble growth factors (Callaway and Martin, 1997), increase mineral retention (Cole et al., 1992) and nutrient digestibility (Wohlt et al., 1991), stabilize ruminal pH, and reduce the incidence of acidosis (Dawson et al., 1990). Comparative effects of YC and ionophores on growth performance and carcass traits are lacking. Therefore, the objective of this study was to evaluate YC as a natural alternative to MON on growth performance, cost of gain (**COG**), and carcass characteristics of finishing heavy-weight yearling beef steers.

MATERIALS AND METHODS

All sampling techniques, animal use, and handling were pre-approved by the Colorado State University Animal Care and Use Committee. Two-hundred fifty-two cross-bred yearling steers (BW = 406 ± 24 kg) were ranked and stratified by coat color, unshrunk BW, and rectal temperatures following in-processing at the Southeast Colorado Research Center (Lamar, CO). Steers were blocked by unshrunk BW and randomly assigned to treatment (n = 7 replicates of 9 steers per treatments). Steers were sorted and housed in dirt surfaced pens (6.1 x 18.3 m) with 3.5 m of linear bunk space and a common water fountain. Body weights were collected on d 0, 28, 56, 84, 110, and 125. Initial and final BW was an average of two-day weights (d -1 and 0; d 124 and 125, respectively). Steers were shipped for harvest on d 125.

Dietary treatment factors were: 1) with or without YC (Diamond V XP™, Diamond V Mills, Inc., Cedar Rapids, IA) and 2) with or without MON (Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN) in finishing diets with 19.7% inclusion of

dried distiller's grains (**DDGS**; DM basis). Both YC and MON were offered in the total mixed ration in place of an equal amount of cornmeal (DM basis; target intake = 2.8 g and 33 mg / kg of DMI, respectively). Each treatment group was offered a transition ration from d 1 through 8, and then was moved to a finishing ration on d 9 through the duration of the trial. All rations during both phases were formulated to be isonitrogenous and isoenergetic. Steers were fed twice daily (approximately 0700 and 1700) at an estimated 110% of the previous day ad libitum intake and feed offered was recorded. A sub-sample was collected and analyzed for nutrient composition by a commercial lab (SDK Laboratories, Hutchinson, KS).

Feed bunks were cleaned and orts were collected on a weekly basis (before the 0700 feeding), weighed, analyzed for DM content, and subtracted from the original feed offered (DM) to determine actual feed intakes. The BW values recorded during each weigh-day were transformed to shrunk BW (**SBW** = BW * 0.96) for analysis. Average daily gain and feed efficiencies were calculated on a live basis for each 28-d period. Incidence and description of morbidity and mortality were recorded. Production costs were calculated for each treatment group for each 28-d period using this formula: Cost / kg of gain = (cost of feed on DM basis) x (feed-to-gain ratio; **F:G**). Carcass measurements were obtained for all steers by a data collection service (Cattlemen's Carcass Data Service, Canyon, TX) at a commercial slaughter facility.

The trial was conducted as a 2 x 2 factorial, completely randomized block design with repeated measures. Pens of steers (or replicates) were treated as the experimental unit. Analysis of variance for each continuous response variable was performed using mixed model procedures of SAS (v9.1.3, Cary, NC). Discrete response variables were analyzed using Glimmix and chi-square frequency procedures in SAS. Where applicable, Tukey's comparison procedure was used to test differences between least squares means if significant (or tendencies of) main effects or interactions were found. Significance was declared at $P \leq 0.05$, and a tendency at $0.05 < P \leq 0.10$.

RESULTS

One steer from the MON treatment was treated and then removed on d 110 for respiratory reasons that resulted in death; this was the only incidence of morbidity or mortality in the study. There were no YC x MON interactions detected for any growth performance traits (Table 1). When BW was analyzed as a repeated measure (data not shown), there was a main effect of day on BW ($P < 0.01$), where all cattle gained weight as the study progressed and YC-fed steers had the lowest over-all BW ($P = 0.049$). Additionally, ADG was lower ($P = 0.028$; Table 1) in steers supplemented with YC. While there was no difference ($P > 0.20$) due to treatment in COG, it was numerically lowest for CON steers with no feed additives ($1.77 / kg of BW gain). Twelfth rib fat thickness and frequency of USDA yield grade (**YG**) 4 and 5 carcasses tended to decrease when steers were fed YC alone, but tended to increase when steers were fed MON (YC x MON, $P = 0.072$ and $P = 0.086$, respectively). Feeding YC was associated with a lower ($P = 0.007$) HCW and a greater ($P = 0.003$) number of carcasses grading Choice (**Ch**). While not significant, YC steers had the highest percentage of YG 1 and 2 (81%) and premium Ch and Ch (67%) carcasses.

DISCUSSION

Effect of Saccharomyces cerevisiae fermentation product. In disagreement with our hypothesis, with the exception of ADG and over-all BW, YC-fed cattle did not significantly outperform the CON or MON cattle. The reason for this is unknown. Previously, Cole et al. (1992) reported no affect on growth performance of calves fed increasing levels of YC in receiving diets. However, when calves were challenged intranasally with infectious bovine rhinotracheitis virus, those calves receiving YC had higher DMI and improved weight gain. Similarly, Phillips and VonTungeln (1985) reported that the addition of YC (Diamond V YC, 50 g·animal^{-1}·d^{-1}) in the receiving ration of feeder calves tended to increase DMI, but had no consistent effect on ADG. The authors from both studies concluded that the YC supplementation seemed to provide a greater benefit in stressed calves. Additionally, Phillips and VonTungeln noted that when YC was added to receiving rations containing MON, ADG was depressed compared to those that did not receive the combination of YC and MON (0.87 and 1.04 kg·animal^{-1}·d^{-1}, respectively). A similar response was seen in the current study; when YC was added to the MON diet (Y x M). Average daily gain was lower than when only MON was included. Conversely, Hinman et al. (1998) reported that the addition of YC in the finishing diet of cross-bred steers increased both ADG and G:F. Schingoethe et al. (2004) reported increased feed efficiency in mid-lactation Holstein cows when 60 g cow^{-1}·d^{-1} YC was offered. Comparatively, in the Schingoethe study, the dosage of YC was higher, as was the percentages of CP and NDF in the diet than in the current study (60 g animal^{-1}·d^{-1}, 17.5 %, and 30.8 % versus 56 g animal^{-1}·d^{-1}, ~13% and ~18 %, respectively). This disparity between study results could be attributed to differences in dosage of YC, concentration of NDF and starch in the diet, and the level of stress experienced by the animals. In the current study, only 1 steer was treated and removed from the study due to health reasons which indicates that cattle were minimally stressed and generally in good health, which could be the explanation for a lack of growth performance response due to the dietary supplements offered.

Carcass data indicates that YC-fed steers were consistently better (numerically) than either CON or MON steers in USDA yield and quality grades, having the highest combined percentage of YG 1 and 2 (81%) and of USDA premium Ch and Ch (67%). It is worth noting that while the carcasses of YC-fed steers were superior in carcass USDA yield and quality grades, they were also the lightest in HCW. These results could be interpreted to suggest that YC steers were more optimally finished at a lower end weight than either the CON or MON-fed steers and may not require as many days on feed. The positive effect of YC on quality grade has been previously

reported (unpublished, Diamond V Mills, 1993). However, the difference in USDA quality grade is not supported by a corresponding difference in either quality or marbling score. It is likely that this discrepancy could be attributed to how similar the carcasses were within the Ch / Select (**Se**) USDA grading assignment. The National Beef Quality Audit (Garcia et al., 2008) reported that 79.96% of carcasses (≥ USDA YG 1) from US fed steers and heifers fall within the Ch / Se USDA quality grading assignment, and that the vast majority of the marbling scores are in the lower grade levels (e.g. low Ch = 64.21%).

An increase in total VFA production and a decrease in ruminal acetate:propionate concentrations has been previously reported when YC was offered to ruminants (Williams et al., 1991; Carro et al., 1992; Erasmus et al., 1992;). While not measured, the authors hypothesize that YC supplementation may have caused an increase in total ruminal VFA production along with a decreased acetate:propionate ratio as compared to CON and MON-fed cattle. Increased VFA production, particularly concentration of propionate, could potentially increase intramuscular fat deposition and be the explanation for the increased number of carcasses that graded Ch or higher from YC-fed steers. Smith and Crouse (1984) reported that acetate will provide 70 – 80% of the acetyl units to in vitro lipogenesis in subcutaneous adipose tissue and only 10 – 25% in intramuscular adipose tissue. Conversely, glucose (made from propionate in the liver) will provide 1–10% of the acetyl units in subcutaneous adipose tissue and 50–75% in the intramuscular depot. Therefore, feeding YC could alter VFA concentrations in such a way that would positively affect marbling and carcass quality.

Effect of Monensin. Feeding MON had no effect on growth or carcass characteristics. While our carcass results are consistent with previous studies, the growth performance results are conflicting. Multiple studies on feeding MON to ruminants have been consolidated (Goodrich et al., 1984; Nagaraja et al., 1997) and indicate that across various diets, types of cattle, and conditions, MON has consistently improved feed efficiency, reduced feed intake, lactic acid production, and the likelihood of bloat, and decreased the incidence of coccidiosis. Perhaps the disparity in growth performance between the current and previous studies could be explained by the mode of action of MON relative to the ingredient composition of the total mixed ration (**TMR**).

Ionophores modify the movement of ions across biological membranes, specifically causing Na entry into cells (Pressman, 1976; Smith and Rosengurt, 1978). The biological response of this action has been previously outlined (Schelling, 1984; Nagaraja et al., 1997). In causing this flux of ions, MON increases the molar proportion of propionate at the expense of lactate and concurrently decreases molar proportions of acetate and butyrate produced in the rumen. An increase in propionate improves energy utilization of MON-fed animals. Consequently, these changes in ruminal fermentation, caused by feeding MON, have been shown to prevent acidosis and bloat. Typically, MON is fed in diets high in rapidly fermentable carbohydrates as a preventative measure against such digestive disturbances. In the current study, the authors decreased the proportion of rapidly fermentable, high-concentrate feedstuffs with DDGS. As already mentioned, DDGS are higher in NDF and lower in starch on a DM-basis than corn (NRC, 2000). Nagaraja et al. (1997) reported moderate to marked inhibition of fiber digestibility when cattle were fed MON. Perhaps the discrepancy between the lack of effect on growth performance in MON-fed steers compared to previous studies could be explained by the inability of MON to be effective when fed in TMR containing distiller's grains. While the inclusion of corn was still relatively high in our study, it was still below industry averages (Vasconcelos and Galyean, 2007). It seems possible that an effect on growth performance due to MON was not detected because some of the starch was replaced with fat by the DDGS, hence the mode of action of MON may have been limited. Recently, Depenbusch et al. (2008) reported that feeding MON to finishing heifers offered no growth performance or carcass advantage when distiller's grains replaced a portion (25%) of the steam-flaked corn in the TMR. Meyer et al. (2009) reported no significant advantage in ADG or carcass traits of steers fed finishing diets containing distiller's grains and supplemented with MON. However, contrary to the current study where cattle fed MON had a numerically lower F:G (data not shown) than CON cattle, Meyer et al. (2009) reported a significant improvement in F:G when MON was fed to cattle consuming 25% wet distiller's grains and 29.75% each of high-moisture corn and dry-rolled corn.

While feeding MON had no effect on carcass characteristics, cattle fed MON had numerically the highest HCW, but also numerically the highest incidence of liver abscesses and number of carcasses to grade select or lower and the numerically the lowest USDA quality and yield grades. Carcass results are in agreement with 228 previous trials that involved 11,274 head of cattle fed MON-containing diets, summarized by Goodrich et al. (1984). The summary indicated that dressing percentage, marbling score, fat depth, quality grade, and yield grade were either not or negatively affected by MON. Additionally, the inclusion of MON has shown no effect on liver abscess incidence in several studies previously summarized by Nagaraja and Chengappa (1998).

Production Costs. The average price for each of the rations (on a DM-basis) was as follows: CON = \$12.61, YC = \$12.59, MON = \$12.51 and Y x M = \$12.66 / cwt of DM (data not shown). The explanation for the price of the CON ration being higher than either the YC or MON rations is due to the average DM of the CON ration was the lowest, therefore increasing the price on a DM-basis. Due to the modest difference in prices, the COG mimicked the ADG response, with lowest COG for the CON steers and greatest COG for the YC steers, yet unlike ADG, this difference was not statistically different. This value appears to be due to the highest numerical value for F:G in the YC-fed steers. The YC-fed cattle cost approximately 5.82% more to feed than MON-fed cattle. Previous work shows that naturally-fed

cattle tend to cost 39% more (Fernandez and Woodward, 1999) and that consumers are willing to pay more for the product (Boland et al., 2002). Should YC be used as a natural alternative to MON for the production of "natural beef," based on this study, the producer would need to be compensated by a market premium of at least 6% if producers were to maintain their profit margin per kilogram of gain. However, it could be elucidated, due to a higher percentage of carcasses from YC-fed cattle that graded USDA YG 1 or 2 and quality grade Ch or better, that cattle could be finished a lower end weights. This would allow for fewer days on feed and lower total production costs.

Summary and Implications. In conclusion, there seems to be some benefit, particularly from a carcass merit stand-point, from feeding YC. Our data indicate that the response of feedlot cattle to MON was limited in steers consuming a diet with 19.7% (DM basis) of DDG. Therefore, because few differences were detected due to either YC or MON treatment, the results of the current study indicate that the conditions were favorable for "naturally-fed" cattle to perform well without any feed additive. What may have been novel in this study was good bunk management, ration design, weather, and a subset of cattle that would allow for abandonment of feed additives. It could also be argued that the implanting that occurred before the cattle were selected to be in this study may have impacted the results in such a manner that differences due to treatment could not be detected in some cases. Admittedly, while the finishing phase of this trial was designed to emulate natural-beef feeding practices for the CON and YC group, it would have been ideal for the steers to have not received implants before the trial. Regardless, the authors feel that further research is needed for determination of effective dosage level of YC in finishing beef rations dependent upon varying levels of roughage and starch. Feeding YC may render more benefit in growth performance if fed at a higher dosage level or when fed to cattle under different feeding and management strategies.

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Table 1. Growth performance and carcass characteristics of pens of steers fed diets supplemented with or without *Saccharomyces cerevisiae* fermentation product and with or without an ionophore (n = 7)[1]

	Treatment					P-value		
Item	CON	YC	MON	Y x M	SE	YC	MON	Y x M
Number of Steers[2]	63	63	62	61	-	-	-	-
Number of Pens	7	7	7	7	-	-	-	-
Performance								
Initial BW[3], kg	391.1	391.9	392.9	391.3	8.07	0.956	0.944	0.885
Final live BW[3], kg	603.0	590.1	603.0	592.3	7.91	0.109	0.877	0.882
DMI, kg	10.38	10.17	10.43	9.72	0.30	0.122	0.487	0.385
ADG, kg	1.679[a]	1.544[b]	1.616[a]	1.574[b]	0.04	0.028	0.677	0.238
G:F	0.165	0.154	0.157	0.165	0.01	0.823	0.771	0.142
COG[4]	1.77	2.00	1.89	1.85	0.11	0.407	0.916	0.209
Carcass traits								
Hot carcass wt, kg	373.5[a]	363.3[b]	375.3[a]	368.3[b]	5.11	0.007	0.278	0.613
Dressing percentage	61.97	62.27	61.60	62.14	0.35	0.494	0.250	0.743
Longissimus muscle area, cm^2	92.2	89.1	92.2	91.5	1.54	0.216	0.454	0.435
KPH fat, %	1.855	1.896	1.857	1.890	0.05	0.421	0.945	0.922
12-th rib fat, cm	1.176	1.102	1.047	1.142	0.05	0.820	0.349	0.072
USDA yield grade, calculated	2.59	2.58	2.46	2.55	0.10	0.647	0.340	0.593
USDA yield grade 1,[5] %	18	15	5	5	-	0.860	0.514	0.831
USDA yield grade 2,[5] %	58	66	62	56	-	0.869	0.703	0.540
USDA yield grade 3,[5] %	23	19	18	19	-	0.756	0.685	0.652
USDA yield grade 4 and 5,[5] %	2	0	0	5	-	0.386	0.386	0.086
Marbling score[6]	SM^{11}	SM^{22}	SM^{08}	SM^{07}	10.48	0.635	0.357	0.515
Quality score[7]	388	398	387	391	5.92	0.229	0.418	0.617
USDA Premium Choice,[4] %	10	12	15	3	-	0.239	0.659	0.155
USDA Choice,[5] %	45[b]	55[a]	30[b]	58[a]	-	0.003	0.364	0.152
USDA Select,[5] %	45	33	53	38	-	0.119	0.393	0.782
USDA Standard,[5] %	0	0	2	0	-	0.328	0.328	0.328
Liver abscesses,[5] %	18	23	31	20	-	0.603	0.363	0.115

[1] Diets consisted of a basal control (**CON**) diet, supplemented with or without *Saccharomyces cerevisiae* fermentation product (YC; Diamond V® "XP", Diamond V Mills, Inc., Cedar Rapids, IA) and with or without an ionophore (**MON**; Rumensin®, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and the two-way interaction of yeast x ionophore (**Y x M**).

[2] On d 110 of the trial, 1 steer was removed from the study for respiratory problems that lead to mortality. Also, 2 carcasses were not accounted for from carcass data collection service in Y x M group.

[3] The BW values are reported as shrunk BW (**SBW** = BW * 0.96). Initial BW was based on an average of both d -1 and 0 values, interim BW's were collected on days 28, 56, 84, 110 (data not shown), and final live BW was based on an average of both d 124 and 125. When BW was analyzed as a repeated measure (data not shown), there was a main effect of day on BW ($P < 0.01$) where all cattle gained weight as the study progressed. There was not a Y x M interaction detected for BW ($P = 0.678$), however YC-fed steers had lower over-all BWs ($P = 0.049$) than either CON or MON steers.

[4] Cost / kg of gain (**COG**) = (cost of feed on DM-basis) x (feed-to-gain ratio).

[5] Data analyzed using Chi-square test; no SEM available.

[6] SM = Small = 400.

[7] Select = 300-399.

[a,b] Least squares means without a common superscript differ, $P \leq 0.05$.

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THE EFFECTS OF CO-ENSILING WET DISTILLER'S GRAINS PLUS SOLUBLES WITH HAYLAGE ON FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF FINISHING STEERS

R. P. Arias[1], L. J. Snyder[1], R. P. Lemenager[2], and S. L. Lake[3]
Departments of [2]Animal Sciences and [1]Agronomy, Purdue University, West Lafayette, IN
[3]Animal Science Department, University of Wyoming, Laramie, WY

ABSTRACT: The objective of this study was to evaluate the effects of co-ensiling wet distiller's grains plus solubles (WDGS) with haylage (H) on feedlot performance and carcass characteristics. Sixty-four Angus-cross steers (328 ± 43kg) were blocked by BW, allotted in 16 pens and randomly assigned to one of four dietary treatments: 1) a corn based finishing diet (**CON**), 2) H co-ensiled with WDGS (**CO-EN**; 3:1 H:WDGS DM basis), 3) H mixed with WDGS at feeding (**H+WDG**), and 4) H mixed with dry distiller's grains plus solubles (DDGS) at feeding (**H+DDG**). All diets were formulated to be isocaloric, isonitrogenous and to meet NRC (1996) requirements. Diets were fed *ad-libitum* until harvest (12^{th} rib fat-depth of 1.07 ± 0.29 cm). Carcass characteristics were measured after a 24-h chill. Subcutaneous fat thickness over the longissimus at the 12^{th} rib (BF) was set to 1.14 cm in all treatments and introduced in the model as a covariate to analyze performance variables (Final BW, BW gain, G:F, days on feed and ADG) and all carcass characteristics variables (except for BF). Steers fed the CO-EN diet had greater (P < 0.01) DMI and ADG compared to other treatments. Steers fed the CON diet required greater (P = 0.02) days on feed to reach harvest compared to CO-EN, H+WDG and H+DDG. There were no differences in final BW (P = 0.11), HCW (P = 0.15), dressing % (P = 0.21), KPH % (P = 0.61) or yield grade (P = 0.11) among treatments; however, BW gain tended to be greater (P = 0.09) in CON and CO-EN steers compared with H+WDG. Ribeye area tended to be greater (P = 0.09) in CON steers when compared to H+WDG. Steers fed the CON diet had higher marbling scores (P < 0.01) and quality grade (P = 0.04) compared to H+WDG, with CO-EN and H+DDG steers being intermediate. These data suggest that co-ensiling H with WDGS results in equal or greater performance, and similar carcass characteristics, when compared to corn based diets and those where WDGS and DDGS were added at the mixer.

Keywords: Finishing steers, WDGS, Co-ensiled.

Introduction

The beef industry serves as one of the most important value-added enterprises in the U.S. with over a million farms and ranches benefiting directly from the sales of cattle (NCBA, 2000). Interestingly, small and medium-sized beef producers account for 96.5% of the beef operations (USDA, 1997). According to the NCBA (2008), record large total meat supplies in the U.S. will affect the demand of beef and limit prices through 2008, and at the same time record high demand for corn by ethanol plants will support higher prices of corn for the foreseeable future. These conditions, combined with drought, increasing competition for land, as well as, the recent downturn in the economy, will continue limiting all producers, but especially the competitiveness of the small and mid-sized beef feedlot operations. Therefore, strategies focused on reducing costs via improving production efficiency have become increasingly more important. The majority of feedlots feed finishing diets consisting of more than 80% cereal grains (Bertram et al., 2004); thus, feed costs represent the largest single cost item in most beef operations. Proper utilization of the ethanol industry co-products in the form of distiller's grains (wet and dry) has resulted in equal, and sometimes greater, performance of feedlot cattle when fed at levels to supply adequate protein and energy, replacing a portion of corn with distiller's grains (Gordon et al., 2002; Pingel and Trenkle, 2006; Gunn et al., 2008). However, the high energy costs incurred to eliminate moisture from wet distillers grain (WDGS; ~30% DM) to convert them to dry distillers grains (DDGS; ~90% DM) brings the price of DDGS close to the price of corn. On the other hand, even though WDGS are cost effective, they have two major disadvantages that impede efficient utilization in small and mid-size feedlots: 1) high transportation cost, due to lower amounts of dry matter per volume and 2) a short shelf-life (3-7 days depending on relative moisture and temperature). However, ensiling or co-ensiling WDG with other ingredients have been reported. Garcia and Kalscheur (2004) reported successful storage and co-ensiling of WDG with corn silage, soybean hulls, and wet beet pulp. Similarly, Arias et al. (2008) reported equal or better performance of pregnant heifers fed co-ensiled corn silage with WDGS when compared to traditional corn based diets and corn silage rations mixed with DDGS or WDGS at feeding. Co-ensiling (in this study) refers to the process of ensiling fresh-cut corn plants mixed with WDGS. We hypothesize that feeding feedlot cattle diets containing corn silage and WDGS co-ensiled together, would result in feedlot performance and carcass quality similar to control fed cattle. Additionally, co-ensiling will represent an advantage for producers in terms of creating the capability to transport greater amounts of WDGS at once, and store them without spoilage risk. Therefore, the objective of this study was to evaluate the effect of co-ensiling haylage with WDGS on feedlot performance and carcass quality of finishing steers, by comparing the co-ensiled product to a traditional corn based diet and two corn silage diets plus DDGS or WDGS added at the mixer immediately prior to feeding.

Materials and Methods

Animals. Sixty-four Angus-cross finishing steers (average initial BW of 328 ± 43 kg) were used in a randomized complete block design. Steers were weighed in two consecutive days prior to the start of the study, blocked by weight, allotted in 16 pens (4 replicas/treatment), and assigned to one of four dietary treatments to examine the effects of co-ensiling WDGS with haylage, all diets were formulated be isonitrogenous and isocaloric, and to meet or exceed NRC (2000) requirements for finishing steers. Weight was recorded every 28 days to monitor performance. To compare carcass quality across treatments, steers were harvested individually when they reached a 12th rib fat-depth of approximately 1.14 cm. However, no individual steer was allowed to remain alone in a pen, thus, if three out of four steers had reached a 12th rib back fat depth of 1.14 cm, all steers in that pen were harvested. A B-mode ultrasound (Aloka American, Ltd., Wellington, CT) was utilized every 28 days to evaluate 12th rib fat thickness during the first 90 days of the experiment and every 15 days from d. 90 to d. 181. Final weights were an average of pre-feeding weights taken on two consecutive days before shipping to a commercial beef packing plant. Carcass data was collected by trained plant personnel following a 24-hour chill (except for hot carcass weights that were taken immediately after exsanguinations) and included: 1) subcutaneous fat thickness over the longissimus at the 12th rib; 2) rib-eye area at the 12th rib; 3) KPH fat as a percentage of carcass weight; 4) dressing percent; 5) USDA yield and quality grades; and 6) marbling score. Two steers from the H+WDG treatment suffered non-treatment related injuries during the trial and were excluded from the study. All procedures for this experiment were approved by the Purdue University Animal Care and Use Committee.

Diets. Steers were fed once daily at 0900 and allowed *ad-libitum* access to feed and water. Dietary treatments consisted of: 1) a corn based finishing diet (CON); 2) haylage co-ensiled with WDGS (CO-EN; 3:1 Haylage:WDGS on a DM basis); 3) haylage mixed with WDGS at feeding (H+WDG); and 4) haylage mixed with DDGS at feeding (H+DDG). Haylage used for this experiment consisted of 40% Tall Fescue, 20% Orchard Grass, 20% Red Clover and 20% others, on a DM basis. Minerals (46% fine ground corn, 40% $CaCo_3$, 8.0% inorganic mix, 6.0% urea) were supplemented at a rate of 230 g/head/day during the length of the study.

Statistical analysis. Steers performance and carcass characteristics data were analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) for a completely randomized block design with pen as the experimental unit. Barn and treatment effects were pooled and included in the model. Significant means ($P < 0.05$) were separated using LSD. Means with an F-test of $P \leq 0.10$ were considered a tendency. Subcutaneous fat thickness over the longissimus at the 12th rib (BF) was set to 1.14 cm in all treatments, and introduced in the model as a covariate to analyze performance variables (Final BW, BW gain, G:F, days on feed and ADG) and all carcass characteristics variables (except for BF). It should be noted that once the covariate (BF) is included in the model to adjust these variables, the reported values become predicted values.

Results and Discussion

Descriptive statistics for performance and carcass characteristics data are presented in Table 3 and 4 respectively.

Performance. By design, there were no differences ($P = 0.99$) in initial BW across treatments. DMI was greatest for the CO-EN steers, intermediate for the H+DDG fed steers, and lowest for CON fed steers ($P < 0.01$). The higher DMI for CO-EN steers may be attributable to diet acceptability, since the co-ensiled product proved to be more stable, with pH readings near or below 4.0 which significantly slows or inhibits yeast, mold and fungal growth (Buckmaster, 2008). In addition, Schmelz et al., (2008) found similar effects on DMI using WDGS co-ensiled with whole plant chopped corn in dairy cows. There were no differences ($P = 0.11$) between treatments for final BW, but BW gain tended ($P = 0.09$) to be greater for steers fed the CON and CO-EN diets compared to steers fed the H+DDG diet. Feed efficiency (G:F) was greater ($P = 0.01$) for the H+WDG fed steers compared to other treatments. Steers fed the CON diet were on feed longer ($P = 0.02$) than any other treatment. Daily gain was greatest ($P < 0.01$) in CO-EN fed steers, intermediate for steers fed the H+WDG and H+DDG fed steers and lowest for CON fed steers. Daily gains followed a similar pattern to DMI. Arias et al. (2008) reported similar results in terms of G:F and ADG when evaluating co-ensiled corn with WDGS in pregnant heifers on a limit-fed scheme. Trenkle (2007) also reported greater gains with WDGS plus corn or hay when comparing them with corn based diets. The improved responses observed with the diets containing distillers grains could be the result of reduced negative associative effects interrelated with supplementing fiber with starch (Trenkle, 2007).

Carcass characteristics. There were no differences in HCW ($P = 0.15$), KPH, ($P = 0.61$), dressing % ($P = 0.21$), or yield grade ($P = 0.11$) among treatments. Ribeye area tended to be greater ($P = 0.10$) in CON steers when compared to H+WDG. Steers fed the CON diet had higher marbling scores ($P = 0.01$) and quality grades ($P = 0.04$) than H+WDG steers with the CO-EN and H+DDG steers being intermediate. These results are similar to those presented by Reinhardt et al. (2007) and Gunn et al. (2008) who reported a decline in marbling scores when distiller's grains were included at 23% or more of the DM. Greater REA and marbling scores in the CON fed steers could also be a result of longer days on feed.

Implications

These data suggest that co-ensiling haylage with WDGS results in equal or greater overall performance, and similar carcass characteristics, when compared to corn based diets

and those where WDGS and DDGS were added at the mixer.

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Table 1. Ingredient composition of steer diets.

Ingredient	Diets[1] (% of DM)			
	CON	**CO-EN**	**H+WDG**	**H+DDG**
Haylage[2]	—	—	11.5	11.6
Corn silage	16.6	—	—	—
Cracked corn	69.2	64.7	61.6	61.8
Soybean meal	14.2	—	—	—
Co-ensiled[3]	—	35.3	—	—
WDG[4]	—	—	26.9	—
DDG[5]	—	—	—	26.6

[1] CON = corn silage with soybean meal, CO-EN = co-ensiled haylage with WDGS, H+WDG = Haylage plus WDGS added at mixing, H+DDG = haylage plus DDGS added at mixing.
[2] Haylage: 30.6% DM, 17.9% CP, 43.6% NDF (DM basis).
[3] Silage mix of haylage and WDGS on a 3:1 DM basis.
[4] WDG = Wet distillers grains plus solubles.
[5] DDG = Dry distillers grains plus solubles.

Table 2. Formulated composition of steer diets (DM basis).

Ingredient	Diets[1,2]			
	CON	**CO-EN**	**H+WDG**	**H+DDG**
DM, %	73.0	65.0	59.0	74.0
CP, %	14.6	14.4	14.8	14.8
NDF, %	15.7	21.4	22.5	22.5
NEg, Mcal/Kg.	1.46	1.48	1.46	1.46
TDN, %	86.8	87.1	87.1	87.1

[1]CON = Haylage plus soybean meal and cracked corn; CO-EN = co-ensiled haylage with wet distiller's grains 3:1 (DM basis), H+WDG = Haylage plus wet distiller's grains with solubles added at mixing, H+DDG = haylage plus dry distiller's grains with solubles added at mixing.
[2]Dietary energy and protein were formulated using tabular values (NRC, 1982).

Table 3. Effect co-ensiled wet distillers grains with haylage on growth performance of Angus finishing steers.

Variables	Treatments[1]					
	CON	CO-EN	H+WDG	H+DDG	SEM[2]	P value
DMI (kg/day)	8.18[c]	10.46[a]	8.57[c]	9.64[b]	0.32	<0.001
Initial BW (kg)	328.9	329.0	323.6	329.1	12.1	0.998
Final BW (kg)[3]	557.9	563.4	528.5	563.4	13.6	0.108
BW Gain (kg)[3]	230.3[d]	234.4[d]	200.6[e]	214.1[de]	11.5	0.093
G:F[3]	0.19[b]	0.19[b]	0.24[a]	0.18[b]	0.01	0.006
Days on Feed[3]	161[a]	128[b]	119[b]	129[b]	6.7	0.015
ADG (kg/day)[3]	1.45[c]	1.83[a]	1.68[b]	1.68[b]	0.05	<0.001

[1] CON = Haylage plus soybean meal and cracked corn; CO-EN = co-ensiled haylage with wet distiller's grains 3:1 (DM basis), H+WDG = Haylage plus wet distiller's grains with solubles added at mixing, H+DDG = haylage plus dry distiller's grains with solubles added at mixing.
[2] The greatest SEM is presented (n = 4 pens/treatment).
[3] Means adjusted by covariate for subcutaneous fat thickness over the longissimus at the 12th rib set at 1.14 cm for all treatments.
[a,b,c] Means within a row lacking a common superscript differ ($P \leq 0.05$).
[d,e] Means within a row lacking a common superscript tend to differ ($P \leq 0.10$).

Table 4. Effect of co-ensiled wet distillers grains with haylage in carcass characteristics of Angus finishing steers.

Variables[2]	Treatments[1]					
	CON	CO-EN	H+WDG	H+DDG	SEM[4]	P value
Hot Carcass Wt (kg)	343.9	349.6	329.1	334.3	8.8	0.153
Fixed Back Fat (cm)[2]	1.14	1.14	1.14	1.14	n/a	n/a
Real Back Fat (cm)[3]	0.95	1.14	0.96	1.21	0.08	0.017
Rib Eye Area (cm^2)	87.7[d]	80.6[de]	75.5[e]	81.2[de]	3.2	0.095
KPH (%)	2.06	2.13	2.16	2.45	0.13	0.608
DP (dressing %)	62.9	64.1	64.4	63.6	0.5	0.207
Yield Grade	2.57	2.98	3.06	2.88	0.14	0.109
Marbling[5]	598[a]	556[ab]	528[b]	570[ab]	20	0.012
Quality Grade[6]	17.5[a]	17.2[ab]	16.8[b]	17.2[ab]	0.2	0.044

[1] CON = Haylage plus soybean meal and cracked corn; CO-EN = co-ensiled haylage with wet distiller's grains 3:1 (DM basis), H+WDG = Haylage plus wet distiller's grains with solubles added at mixing, H+DDG = haylage plus dry distiller's grains with solubles added at mixing.
[2] Means adjusted by covariate for subcutaneous fat thickness over the longissimus at the 12th rib set at 1.14 cm for all treatments.
[3] Fat thicknesses means of steers; included in table to support the use of the covariate.
[4] The greatest SEM is presented (n = 4 pens/treatment).
[5] Marbling score: 400 = Slight 0, 450 = Slight 50, 500 = Small 0, etc.
[6] Quality grade: 15 = Select$^-$, 16 = Select$^+$, 17 = Choice$^-$, 18 = Choice$^+$, etc.
[a,b,c] Means within a row lacking a common superscript differ ($P \leq 0.05$).
[d,e] Means within a row lacking a common superscript tend to differ ($P \leq 0.10$).

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PASTURE PRECONDITIONING CALVES AT A HIGHER RATE OF GAIN IMPROVES FEEDLOT HEALTH BUT NOT POST-WEANING PROFIT

C.P. Mathis, S. H. Cox, C. A. Löest, M. K. Petersen, and J. T. Mulliniks
[1]New Mexico State University, Las Cruces, NM, 88003

ABSTRACT: Preconditioning (**PRECON**) beef calves on the ranch of origin reduces post-shipping health risk. Previous research suggests pasture PRECON can improve subsequent feedlot health and profit of calves compared to drylot PRECON. However, few comparisons of on-ranch pasture PRECON methods exist. This study used 132 steers (218 ± 12 kg avg. initial BW) over 2 yr to compare the impact of low (**LOW**) and high (**HIGH**) input pasture PRECON methods on performance and profit during the PRECON (weaning to 49 to 53 d) and finishing (**FINISH**; end PRECON to slaughter) phases. At weaning (d 0), steers were randomly assigned to LOW or HIGH treatments. Steers were fenceline-weaned for 7 d beginning d 0, then transported to their respective pastures (d 7). During PRECON, the HIGH steers had ad libitum access to a corn/wheat midds-based pellet in a self feeder, and LOW steers were supplemented with a 32% CP range cube (0.57 kg/d; 3×/wk). The HIGH steers gained 0.32 kg/d more ($P < 0.01$; 0.50 vs. 0.82 kg/d) than LOW steers, resulting in a 19 kg heavier final PRECON BW for HIGH steers ($P < 0.01$; 242 vs. 261 kg). The LOW steers had a $21/hd lower final PRECON value ($P < 0.01$; $559 vs. $580), but this difference was offset by a $42/hd lower feed cost ($P < 0.01$; $10 vs. $51). Consequently, LOW steers had a net income advantage of $21/hd ($P < 0.01$) during PRECON. Following PRECON, steers were finished at a commercial feedlot where they exhibited no differences in ADG, final BW, or carcass characteristics ($P \geq 0.28$). Morbidity during FINISH was 16.7 percentage units higher for LOW steers ($P = 0.01$; 24.6% vs. 7.9%), resulting in a $6.63/hd greater medicine cost than HIGH steers ($P = 0.05$; $11.41 vs. $4.51). There was no impact of PRECON treatment on FINISH net income ($P = 0.49$), nor on profit from weaning to harvest ($P = 0.90$). In conclusion, pasture PRECON steers at a higher rate of gain can improve FINISH health, but because of the higher PRECON cost, may not increase post-weaning profitability achieved by PRECON at a lower rate of gain.

Key Words: Preconditioning, Beef Calves, Feedlot

Introduction

Guidelines for adding value to calves through a defined vaccination protocol and the separation of weaning and shipping by 45 d or more (i.e., VAC-45; Anonymous, 2005) are justified by improved subsequent performance and health (Cravey, 1996; Lalman et al., 2005). Industry acceptance of such guidelines is evident in that more than 25% of the reported calves sold through Superior Livestock video auction in 2007 were marketed as "VAC-45" (King, 2007). However, management intensity and cost during preconditioning can vary. Mathis et al. (2008) demonstrated that a low-input pasture preconditioning approach can cost as much as $52/hd less than a high-input drylot approach. Furthermore, Boyles et al. (2007) and Mathis et al. (2008) showed that compared to drylot preconditioning, pasture-based approaches can yield improved health during the finishing phase.

Controlled experiments comparing pasture-based preconditioning methods on calf performance and profitability through harvest are limited. Therefore, this study compared pasture preconditioning methods with a high level of nutritional input to a lower input approach to evaluate performance and profit from weaning through harvest.

Materials and Methods

Over 2 yr, 132 calves (218 ± 12 kg avg. initial BW) were used to compare two preconditioning methods at the New Mexico State University Corona Range Livestock Research Center (**CRLRC**) located 13 km east of Corona, NM (avg. elevation = 2,000 m; avg. annual precipitation = 380 mm). All animal handling and experimental procedures were in accordance with guidelines established by the New Mexico State University Animal Care and Use Committee. Calves originated from the CRLRC spring-calving British cross cow herd, and were born in February, March, or April. Steer calves were castrated at branding in early May, and were weaned in mid-September.

Calfhood Vaccination Protocol. At branding, and on d 1 and d 28 post-weaning, calves were vaccinated against bovine respiratory syncytial virus, infectious bovine rhinotracheitis, bovine viral diarrhea, and parainfluenza 3. Calves were also administered a 7-way clostridial vaccine at branding and on d 28, and were vaccinated against Pasteurella on d 1 post-weaning.

Preconditioning Phase. All preconditioning period BW were measured unshrunk between 0900 and 1100 h, and a 3% pencil shrink was applied. At weaning (d 0), calves were weighed, assigned a market price, and randomly assigned to one of two preconditioning nutrition treatments: 1) ad libitum self-fed pellets (**HIGH**) or 2) hand-fed range cubes (0.57 kg hd^{-1} d^{-1}; **LOW**). The LOW treatment was designed to be similar to the low-input pasture treatment evaluated in our previous work (Mathis et al., 2008). In addition, the self-fed preconditioning pellet (Table 1) offered to the HIGH steers was formulated identical to the pellet fed by Mathis et al. (2008) to steers preconditioned in

a drylot; such that the main difference in the high-input confinement feeding approach previously employed and the current HIGH treatment was the environment (pasture vs. confinement). Treatments were replicated within year.

Steers were fenceline-weaned for 7 d beginning on d 0. On d 0 to 6, alfalfa hay (1.13 $kg \cdot hd^{-1} \cdot d^{-1}$) was placed in and around self feeders to accustom all calves to the self feeder. Calves were transported to their respective treatment pasture on d 7. The same four native range pastures (minimum 4.1 ha/hd) were used each year. Native range pastures were not grazed during the spring and summer growing season before stocking during preconditioning. Forage availability exceeded cattle need each yr. To estimate forage quality, grass samples were annually hand-plucked from each pasture at the beginning (6.0% CP, 70.8% NDF, and 44.2% ADF) and end (4.6% CP, 69.4% NDF, and 45.6% ADF) of preconditioning. Free choice access to water and a loose mineral mix (38% NaCl, 12% Ca, 8% P, 2% K, 2% Mg, 2500 ppm Mn, 1000 ppm Cu, 1000 ppm Zn, 13 ppm Se, and 125,000 IU/kg Vitamin A; Hi-Pro Feeds, Friona, TX) was provided.

On d 8 to 10 after weaning, LOW calves were trained to hand-delivery of protein supplement by enticement with 1.13 $kg \cdot hd^{-1} \cdot d^{-1}$ alfalfa hay, plus protein supplementation with 0.57 $kg \cdot hd^{-1} \cdot d^{-1}$ of a hand-fed 32% CP range cube. Beginning d 11, supplementation frequency was reduced to 3×/week. Calves were fed between 1000 and 1200 h.

Steers on HIGH were given immediate ad libitum access to a preconditioning pellet in a self-feeder. A single self feeder was placed near water in each HIGH pasture. On d 7 to 9, 1.13 kg $hd^{-1} \cdot d^{-1}$ of alfalfa hay was placed on top of the self-fed pellets to initiate pellet consumption. Average daily pellet intake was 4.21 ± 0.35 kg. During yr 2, three steers in one HIGH treatment group escaped the treatment pasture during preconditioning and therefore were removed from the experiment.

Each year BW was measured on d 28 and at the end of the preconditioning phase (d 53 in yr 1; d 49 in yr 2). The day final preconditioning BW was measured marked the end of the preconditioning phase. All steers were held overnight in a common drylot and fed 4.54 kg/hd alfalfa hay, then shipped to the feedyard the following morning. Steers remained at the CRLRC for at least 49 d post-weaning and conformed to guidelines for Value Added Calf-45 (VAC-45) weaning option (Anonymous, 2005).

Weaning price and final preconditioning price was individually applied to each calf based upon prices in the New Mexico Weekly Weighted Average Feeder Cattle Report (USDA CV LS795) for the beginning and ending week of the preconditioning phase. No premium for preconditioning was applied. Purchased feed price/ton for yr 1 and 2, respectively, were $224 and $206 for preconditioning pellets, $250 and $242 for range cubes, and $165 and $200 for alfalfa hay. Feed costs were applied as weight of feed delivered to each pasture times unit feed cost.

Finishing Phase. After preconditioning steers were shipped to a commercial feedlot (yr 1, Double A Feeders, Clayton, NM; yr 2, Celebrity Feeders, Felt, OK). Final BW and price of steers from the preconditioning phase was used as the initial BW and price of steers for the finishing phase.

Steers were received at the feedlot in early November, and were managed according to standard procedures in place at the respective feedlot. Feedlot management observed steers at arrival, considered the previous health and management protocol, and subjectively decided the receiving preventive pharmaceutical and growth-promoting implant protocols for all steers. Diagnosis of morbid was based on subjective visual appraisal by experienced feedlot staff. Steers were housed in pens allowing more than 9.3 m^2/hd and 40 cm/hd linear bunk space.

When steers were processed for secondary application of growth-promoting implants in yr 1, they were also weighed and individually assigned to marketing groups using the ultrasound technology and computer software of the Cattle Performance Enhancement Co. (CPEC, Oakley, KS). Once the optimum individual market date was estimated, steers were assigned to marketing groups harvested between March and early July. In yr 2, all steers were visually appraised by experienced feedlot staff to determine a single marketing date (June 3, 2008) to achieve optimal performance. In yr 1, steers averaged 181 ± 1.37 DOF; whereas in yr 2 steers were fed for 209 d. Steers were harvested at a commercial facility (National Packing Co., Liberal, KS). Hot carcass weight (HCW) was collected at slaughter, and longissimus muscle area, fat thickness, and marbling score were evaluated by an independent data collection service (Cattle Trail LLC, Johnson, KS) after carcasses were chilled. Carcasses were sold on an individual basis through the National Beef Grid, with premiums and discounts applied using HCW and USDA quality and yield grade.

Statistical Analysis. The effect of preconditioning treatment on performance, carcass, and financial data was evaluated using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with yr as a blocking factor and pasture as the experimental unit. The model included replicate, yr, and treatment. Chi-square in the FREQ procedure of SAS (SAS Inst. Inc., Cary, NC) was utilized to evaluate the categorical distribution of USDA quality grade, USDA yield grade, morbidity, and death loss.

Results and Discussion

Preconditioning Phase. There were no differences in weaning BW, price, nor value at the beginning of the treatment period ($P \geq 0.14$; Table 2). Interim BW (d 28) and ADG from weaning to d 28 were similar ($P \geq 0.22$); however, HIGH steers were 19 kg heavier ($P < 0.01$) than LOW at the end of preconditioning because they gained 0.32 kg/d more ($P < 0.01$) across the entire preconditioning period. The BW advantage of HIGH steers occurred primarily because ADG from d 28 to the end of preconditioning was more than 3-fold higher ($P < 0.01$) for HIGH than LOW. Treatment also yielded dissimilar patterns of ADG during preconditioning. Specifically, ADG among HIGH steers was higher from d 28 to the end of preconditioning than during the initial 28 d; likely resulting from increasing intake of self-fed pellets (not measured by period) during the latter portion of preconditioning. In contrast, the rate of gain observed among LOW steers after d 28 was 62% less than that exhibited during the first 28 d

of preconditioning. This outcome is similar to previous work (Mathis et al., 2008) where calves managed in an almost identical pasture preconditioning treatment gained 58% less during the second half of preconditioning. Mathis et al. (2008) attributed this observation to declining quality of forage during the treatment period, which is supported by 1.4% average decline in forage CP content from September (6.0%) to November (4.6%) in the current study. The BW advantage of HIGH steers at the end of preconditioning was reflected in a $4.10/45.4 kg lower ($P < 0.01$) final preconditioning price, but more than $20/hd greater ($P < 0.01$) final preconditioning value than LOW steers.

By design, preconditioning feed cost was lower ($41.57/hd; $P < 0.01$) for LOW than HIGH steers. Even though HIGH steers generated more gross income during preconditioning, the 5-fold higher cost of the self-fed pellet feeding method resulted in a $20.54 preconditioning net income advantage ($P < 0.01$) to LOW steers. These results are in agreement with previous work (St. Louis et al., 2003; Mathis et al., 2008) demonstrating that lower cost preconditioning approaches can generate greater net income during preconditioning.

Both treatments yielded monetary losses. The two main factors contributing to these losses were that: 1) a price premium was not applied to preconditioned calf prices, which approximated $8/45.4 kg in 2006 and 2007; and 2) the local market seasonal decline in price from September to November averaged more than $18/45.4 kg during 2006 and 2007 (250-kg calf basis; USDA CV LS795), approximately 2.25 times larger than the United States 5-yr average (2004-2008) seasonal decline of $8.15/45.4 kg from September to November (CattleFax, 2009). Nonetheless, a price premium of $12.45 and $15.12/45.4 kg at the end of preconditioning would have been required for LOW and HIGH, respectively, to monetarily break even during the preconditioning phase.

Finishing Phase. During finishing there were no differences ($P \geq 0.35$) in ADG or final BW, and preconditioning method had no impact ($P \geq 0.42$) on HCW, fat thickness, longissimus area, or marbling score. The distribution of USDA quality grade and yield grade were also similar ($P \geq 0.28$; data not shown). The lack of impact of preconditioning method on carcass attributes and performance measured across the entire finishing period agrees with previous findings (Mathis et al., 2008) that showed no differences when divergent preconditioning methods were compared.

The proportion treated for sickness during finishing was 16.7 percentage units higher ($P = 0.01$) for LOW steers, and resulted in $6.63/hd greater ($P = 0.05$) medicine cost than HIGH. Our previous work (Mathis et al., 2008) showed higher feedlot mortality (7.6 percentage units) among drylot than pasture preconditioned steers. Higher mortality and the numerically greater (14 percentage units) morbidity among drylot preconditioned calves in that study were attributed to the additional stressors of greater dietary and environmental change among the steers preconditioned in confinement on a high-grain pellet than pasture preconditioned calves. Although not statistically separable ($P = 0.36$), death loss in the current study for LOW and HIGH steers were 4.4 and 1.6%, respectively. Across both studies, steers fed range cubes on pasture during preconditioning exhibited feedlot morbidity rates of 25 to 34%. On the other hand, feedlot morbidity was 48% when Mathis et al. (2008) fed a preconditioning pellet in confinement, compared to only 8% when the same pellet formulation was consumed ad libitum by steers preconditioned on pasture. Collectively, these studies indicate that providing a higher plain of nutrition to steers during preconditioning in a pasture environment may better precondition calves to cope with the immune challenges associated with shipping to a commercial feedlot.

Preconditioning treatment had no impact on finishing feed cost ($P = 0.63$), but caused a tendency for higher ($P = 0.11$) total feedlot cost for HIGH steers. This occurred largely because of the higher value of HIGH steers at the end of preconditioning, resulting in a higher initial cost of HIGH steers entering the finishing phase. Carcass price and gross income were similar ($P \geq 0.35$). The numerical 2.8 percentage unit difference in death loss between treatments made the greatest contribution to the $43.42 numerical difference in gross income between LOW and HIGH. Overall, preconditioning treatment had no effect ($P = 0.49$) on finishing phase net income.

Although the differences in net income during the finishing phase were not statistically separable, the numerical advantage in profitability among HIGH steers during finishing compensated for the net income advantage of LOW steers during the preconditioning phase. As a result, overall net income from weaning to harvest was similar ($P = 0.90$) for LOW and HIGH steers.

Implications

Beef calves can be preconditioned on pasture with divergent levels of nutritional input and programmed gain. The cost of nutritional inputs has a substantial influence on the profitability of a preconditioning program. Grazing calves on native rangelands at a higher rate of gain can better prepare calves to remain healthy after shipping off the ranch of origin. However, increased feed input costs often required to achieve a higher rate of gain on pasture may not be cost-effective relative to a lower-cost approach if calves are sold after preconditioning, or retained through harvest.

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Table 1. Composition of pellet fed to HIGH steers

Item	Amount
Ingredient, % of as-fed	
Corn, ground	34.7
Wheat middlings	32.0
Soybean hulls	15.0
Cottonseed meal	5.8
Cottonseed hulls	5.0
Molasses	5.0
Calcium Carbonate	1.5
Potassium Chloride	0.5
Salt, Vitamins, Trace Minerals[1]	0.5
Nutrient Concentration	
CP, % of DM	15.8
NE_m, Mcal/kg DM	2.13
NE_g, Mcal/kg DM	1.41

[1]Includes Rumensin-80 at 0.0125 %.

TABLE 2. Post-weaning performance and profit of steers preconditioned on pasture at a high (HIGH) or low (LOW) rate of gain

	Preconditioning Method			
Item	**LOW**	**HIGH**	**SE**	***P***
Number of head	69	63		
Preconditioning Performance[1]				
Weaning BW, kg	217	219	11.9	0.38
Interim BW, kg	236	242	12.2	0.25
Final BW, kg[2]	242	261	7.5	<0.01
ADG, d 0 to Interim, kg/d	0.69	0.78	0.05	0.22
ADG, Interim to Final kg/d	0.26	0.88	0.12	<0.01
Total ADG, kg/d	0.50	0.82	0.07	<0.01
Preconditioning Financial				
Weaning Price, $/45.4 kg	129.13	127.73	3.51	0.14
Weaning Value, $	615.66	615.51	16.94	0.98
Final Price, $/45.4 kg[2]	104.91	100.81	1.59	<0.01
Final Value, $	559.12	579.98	8.66	<0.01
Feed Costs, $	9.83	51.40	2.69	<0.01
Hay	2.69	2.69	-	-
Drylot Pellet	-	44.10	-	-
Range Cube	7.14	-	-	-
Self-Feeder	-	4.61	-	-
Net Income, $	-66.38	-86.92	6.47	0.01
Feedlot Performance				
Final BW, kg[3]	531	540	28.2	0.38
ADG, kg/d	1.47	1.44	0.02	0.35
% Treated for Sickness[4]	24.6	7.9		0.01
% Death Loss[4]	4.4	1.6		0.36
Carcass				
Hot Carcass Weight, kg	338.0	343.1	17.8	0.42
Fat Thickness, cm	1.50	1.47	0.15	0.69
Longissimus Area, sq. cm	77.5	78.6	1.3	0.58
Marbling Score[5]	514	507	23.6	0.63
Feedlot Financial				
Medicine Cost, $	11.14	4.51	3.34	0.05
Feed Cost, $	414.64	418.27	59.06	0.63
Total Cost, $	1017.76	1035.63	62.64	0.11
Carcass Price, $/45.4 kg	147.62	147.40	0.91	0.87
Gross Income, $	1054.80	1098.28	31.3	0.35
Net Income, $	37.04	62.66	43.12	0.49
Net Income (Weaning to Harvest)	-29.34	-24.26	49.07	0.90

[1]A 3% pencil shrink was applied to all BW.
[2]Preconditioning Final BW and price = initial feedlot BW and price.
[3]Feedlot Final BW is an estimate calculated as carcass weight ÷ average dressing % of marketing group.
[4]Chi-square analysis.
[5]Marbling score: Small 00 = 500.

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MARKET COW FEEDING USING DIFFERENT MANAGEMENT STRATEGIES[1]

M. M. Thompson*, C. S. Schauer*, R. J. Maddock†, and C. L. Wright‡
*Hettinger Research Extension Center, North Dakota State University, Hettinger, ND 58639
†Department of Animal Sciences, North Dakota State University, Fargo, ND 58105
‡Department of Animal and Range Sciences, South Dakota State University, Brookings, SD 57007

ABSTRACT: Forty-eight non-pregnant cows (BW = 597±6.7 kg, BCS= 5.71±0.07) were stratified by weight and BCS to 12 pens to investigate the effects of feeding strategy on feedlot performance, carcass characteristics, and economics. Pens (n = 4) were assigned randomly to one of three treatments (**TRT**): corn-mixed hay (**HAY**), barley-barley silage (**SILAGE**), and a self-fed ground diet using a controlled intake system (**LIMIT**). Diets were formulated to provide 1.32 Mcals NE_g/kg and 11.5% CP using mixed hay, barley silage, and a commercial supplement containing soy hulls as roughage sources for HAY, SILAGE and LIMIT, respectively. Cows were weighed and BCS on d 0, 1, 28, 45, 46, 74, 75, 102 and 103. HAY and SILAGE diet samples were collected on d 6, 22, 43, 60, and 74. LIMIT diet samples were collected on d 1, 6, 36, 60, 63, and 74. After 104 d, 14 cows were sold at auction locally (n = 4 for SILAGE and n = 5 for HAY and LIMIT) and the remaining cows harvested at Dakota Premium Foods LLC, South St. Paul, MN. Cow performance, carcass traits, and economic data were analyzed as a completely randomized design (PROC GLM, SAS) with pen serving as experimental unit. HAY and SILAGE gained faster ($P < 0.01$) because LIMIT acclimated slowly to their diet the first 46 d of study, resulting in lower DMI, ADG, and G:F for LIMIT ($P < 0.02$). LIMIT had higher feed costs than HAY and SILAGE ($P = 0.02$). Despite similar final BCS ($P = 0.19$), LIMIT gained the least ($P = 0.04$). Carcass traits and total cow value were similar across TRT for harvested cows ($P = 0.10$). HAY had the lowest breakeven and greatest return for harvested cows ($P \leq 0.02$); however, breakevens and returns for sold cows did not differ across TRT ($P = 0.15$). Although self-fed diets can improve market cow quality, other low cost alternatives require further investigation.

KEYWORDS: cull cows, market cows, self-fed

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Introduction

The sale of market cows (cull cows) can contribute a considerable portion of income (15-30%) to the annual receipts of cow-calf producers (Feuz, 2006). Six to eight million market cows are slaughtered annually, providing a sizeable supply of muscle cuts to the packing industry (Stalcup, 2008). Generally, spring calving market cows are sold in the fall (following weaning and pregnancy checking) at a time when cow supply is large and economic returns are low. Cow-calf producers give little forethought to adding value to market cows before culling. One method of enhancing market cow value is to feed the cows for a short time (60 to 100 days) and then sell the cows when market prices are seasonally higher (Strohbehn et al., 2004; Strohbehn and Sellers, 2002). As well as increasing market value, a feeding period enables cow-calf producers to improve cow carcass quality (Wright, 2005). Moreover, little research has examined the use of self-feeding protocols as a system of adding value to market cows. As volatility continues in the feed ingredient markets and fuel and other production input expenses increase, re-evaluation of market cow feeding strategies and economic profitability is crucial (Niemela et. al., 2008). Our study objective was to investigate the effects of feeding strategy on cow feedlot performance, carcass characteristics and economics. Our hypothesis was that the three feeding strategies would have similar performance and carcass quality, but the self-fed system would have lower feed and labor costs associated with it as compared to the other two systems.

Materials and Methods

Animals and Dietary Treatments. The NDSU Animal Care and Use Committee approved all protocols. Sixty-eight market cows were purchased locally during a two-wk period (Oct. 22 and 29, 2007). After purchase, cows were delivered to the NDSU Hettinger Research Extension Center. On d 0 and 1, purchased cows were weighed, body condition scored on a scale of 1-9 (**BCS**; Herd and Sprott, 1986) evaluated for pregnancy status, temperament and overall health. From this group, 48 non-pregnant cows were selected as study subjects. Cows were vaccinated for respiratory and clostridial diseases, dewormed, and implanted (Finaplex H, Intervet, Millsboro, DE) on d 1. Cows were stratified by weight (BW = 597±6.7 kg) and BCS (5.71±0.07) and allotted to one of 12 pens (4 cows/pen). Pens were assigned randomly to one of three treatments (**TRT**): corn-mixed hay (**HAY**), barley-barley silage (**SILAGE**) and a self-fed ground diet using a controlled intake system (**LIMIT**). Diets were formulated to provide 1.32 Mcals NE_g/kg and 11.5% CP using ground mixed hay, barley silage, and a commercial supplement containing soy hulls as roughage sources for HAY, SILAGE, and LIMIT respectively (Table 1). Alfalfa haylage and soybean meal (47.5% CP) were included in the HAY diet to prevent ration separation and increase CP level. Ground hay was added to the SILAGE diet to

increase ration DM. Four rations of increasing energy density (data not reported) were fed to HAY and SILAGE cows during the first 40 d to acclimate cows to high grain finishing diets. Fence line feed bunks were read daily at 0700 hrs and slick bunk management was used to determine individual pen daily feed allotment. HAY and SILAGE cows were fed once daily at 0900 hrs. Purina Mills® developed the feeding protocol used for the LIMIT cows. LIMIT cows had continual access to self-feeders containing respective diets (Table 1); LIMIT cows were fed small amounts of baled grass hay daily. LIMIT diets were ground on d 0, 8, 22, 36, 42, 45, 47, 53, 63, and 74. All cows had free access to water in ice-free automatic fenceline water fountains and white salt blocks. To prevent estrus, MGA® pellets were added to all diets. Due to deteriorating pen conditions because of inclement weather, all cows were removed from feedlot pens on d 76, commingled into one group, and placed into a larger pen. From d 76-103, cows were fed a mixed ration at 2.6% BW (based on d 75 BW) containing 25% barley silage, 25% ground mixed hay, 22.5% whole barley, 22.6% cracked corn, 1.9% finishing supplement, 2.5% MGA® pellets and 1.9% calcium carbonate (DM basis, 13.7% CP, 1.14 Mcal NE_g/kg) for the last 28 d prior to harvest. This roughage-based diet was fed because of concerns over possible cow lameness and cows going down during the long transport to harvest.

Measurements and Analysis. Cows were weighed and BCS on day 0, 1, 28, 45, 46, 74, 75, 102 and 103. Intial and final BW were determined by averaging two-d unshrunk weights. HAY and SILAGE diet samples were collected on d 6, 22, 43, 60, and 74. LIMIT diet samples were collected on d 1, 6, 36, 60, 63, and 74. Diet samples from the commingled group were collected on d 80, 90, and 100. Diet samples were composited by TRT and analyzed by a commercial laboratory (Midwest Laboratories, Omaha, NE) for nutrient analysis. After the 104 d feeding period, 14 cows were sold at auction locally (n = 4 for SILAGE and n = 5 for HAY and LIMIT, respectively) to evaluate local market prices (Lemmon Livestock Inc., Lemmon, SD, Feb. 13, 2008) for fattened cull cows. The remaining cows (n=33) were harvested at Dakota Premium Foods, LLC, South St. Paul, MN on d 104 and individual carcass data was collected following a 24-hr chill. Economic values for feedstuffs and other service fees were obtained from purchased costs, local cash grain bids and the USDA NASS North Dakota monthly commodity prices (www.nass.usda.gov/nd). Breakeven and closeout information were calculated using the NDSU Extension CalfWEB closeout analysis program (www.chaps2000.com/calfweb/closeout.asp). Cow performance, carcass traits, and economic data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NY) with pen serving as the experimental unit. Carcass data was analyzed similarly, with missing data points from auctioned cows not included in the data set, but with pen still serving as experimental unit. Treatment means are separated by least square means following a protected F-test ($P < 0.05$).

Results and Discussion

Feedlot Performance. Cow feedlot performance is shown in Table 2. One cow (HAY) was removed from the study because of founder (d 57). All performance data from the removed cow was deleted from subsequent performance analyses. Additionally, two cows were treated for foot rot (LIMIT and HAY, d 49 and 55, respectively). Subsequently, veterinary medicine costs did not differ between TRT and averaged $12.15 ± 0.59 per cow ($P = 0.69$; Table 2). HAY and SILAGE cows gained faster ($P < 0.01$) because LIMIT cows acclimated slowly to their diet the first 46 d of study, resulting in lower DMI, ADG, and G:F for LIMIT cows ($P < 0.02$; Table 2). LIMIT cows DMI and ADG increased when the cows consumed the final self-fed diet during Period 2 (Table 2). At the end of 75 d, LIMIT and SILAGE cows had similar DMI, but differed from HAY cows ($P = 0.007$; Table 2). Despite final G:F being similar across TRT ($P = 0.13$), LIMIT cows had the lowest DMI and ADG and the highest feed cost/kg gain ($P < 0.02$; Table 2) at the end of 75 d. Feed costs/kg gained were similar for HAY and SILAGE cows ($P = 0.02$; Table 2). Although final BCS were similar across TRT ($P = 0.19$), HAY cows were the heaviest, SILAGE cows intermediate and LIMIT cows the lightest ($P = 0.02$; Table 2) before commingling. Yardage costs were 20% lower for LIMIT cows as compared to HAY and SILAGE cows ($P < 0.001$; Table 2). Yardage fees were $0.25 hd/d for LIMIT cows and $0.35 hd/d for HAY and SILAGE cows. The use of self-feeders decreased labor and equipment needs from d 0-75 as compared to more traditional feeding methods (totally mixed rations fed by a feeder wagon).

Carcass Traits and Economics. Carcass traits and total cow value were comparable across TRT for harvested cows ($P = 0.10$; Table 3). This may be attributed to greater DMI and compensatory gain exhibited by LIMIT cows during the 28 d prior to harvest as compared to HAY and SILAGE cows. Cows sent to the commercial abattoir received the same price at harvest ($2.38/kg of HCW). The effect of feeding strategy on auctioned cows and closeout returns is reported in Table 3. Initial average value for the cull cows used in this study was $1.00/kg or $596.70/hd. Feeding these cows for an additional 104 days increased the average cow value to $1,009.31/hd ± $16.26 for harvested cows, with no difference between TRT ($P = 0.14$; Table 3). Additionally, cows sold at auction had increased value as well, averaging $958.18/hd, with no difference between TRT ($P = 0.29$; Table 3). Sale cow BW were similar at local auction on sale day ($P = 0.08$; Table 3). HAY cows had the lowest breakeven and greatest return for harvested cows ($P \leq 0.02$; Table 3); however, the breakevens and economic returns for sold cows did not differ across TRT ($P = 0.15$; Table 3). Cows sold at auction received similar market prices across TRT, with cows averaging $1.31/kg ($P = 0.34$; Table 3).

Implications

Despite the increased dietary adjustment time, LIMIT cows gained more efficiently when consuming their final self-fed diet. Even though carcass quality was similar across TRT, improved carcass quality for LIMIT cows may be attributed to the final self-fed ration and the 29 d commingled feeding

period prior to harvest. Although LIMIT cows consumed less feed and had lower labor costs, feed cost of gain was highest for this group. Potential economic returns by feeding market cows will be highly dependent on several factors: availability of local resources, initial cow body condition, feed costs and availability, days on feed and final carcass characteristics. Self-feeders are a viable alternative system of feeding and improving market cow value. Although self-fed diets can improve market cow quality, other low cost alternative require further investigation.

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Table 1. Dietary ingredient and nutrient concentration of HAY, SILAGE, and LIMIT diets

			LIMIT		
			ACCURATION[a]		IMPACT[b]
Item	HAY	SILAGE	d 0-21	d 22-46	d 47-75
Ingredient , % DM					
Alfalfa haylage	8.5	-	-	-	-
Barley silage	-	16.1	-	-	-
Calcium carbonate	0.7	0.7	-	-	-
Whole barley	-	67.2	-	-	-
Cracked corn	71.4	-	34.8	60.8	78.5
Finish supplement[c]	2.0	1.8	-	-	-
Purina supplement	-	-	52.1	26.1	8.7
MGA pellets[d]	2.6	2.4	2.8	2.8	2.8
Ground mixed hay[e]	12.7	11.8	-	-	-
Soybean meal (47.5% CP)	2.1	-	-	-	-
Grass hay	-	-	10.0	10.0	10.0
12:12 mineral	-	-	0.3	0.3	-
Nutrient concentration[f]					
% DM	76.92	69.22	88.19	87.52	85.2
CP, % DM	11.7	14.5	23.7	22.9	14.8
NE_m, Mcal/kg DM	1.80	1.76	2.02	2.05	1.91
NE_g, Mcal/kg DM	1.21	1.19	1.34	1.36	1.28
Ca:P	2.76	2.81	1.56	1.50	1.62

[a]Purina supplement contained 143 mg/kg Monensin sodium.
[b]Purina supplement contained 250 mg/kg Monensin sodium and 99 mg/kg Tylosin phosphate.
[c]Supplement contained 1100 mg/kg Monensin sodium.
[d]Supplement contained 0.00011% Melengestrol Acetate.
[e]Mixed hay composed of equal parts barley and alfalfa-grass hays.
[f]Analytical results are from composited samples.

Table 2. Influence of market cow feeding strategy on feedlot performance and associated costs

Item	HAY[a]	SILAGE[b]	LIMIT[c]	SEM[d]	*P*-value[e]
No. head	16	15	16	-	-
No. pens	4	4	4	-	-
Initial BW, kg	601.6	590.9	597.6	6.7	0.55
Initial BCS	5.68	5.71	5.75	0.07	0.74
Period 1, d 0-46					
46 d gain, kg	84.5[f]	69.8[f]	18.5[g]	12.4	0.01
DMI, kg/d	17.3[f]	12.7[g]	11.6[h]	0.27	< 0.001

ADG, kg·/d	1.86[f]	1.61[f]	0.40[g]	0.27	0.01
G:F	0.11[f]	0.13[f]	0.03[g]	0.02	0.02
Feed cost/kg gain, $/kg	1.73	1.82	4.86	3.17	0.74
Period 2, d 47-75					
29 d gain, kg	54.7	71.6	73.5	6.6	0.14
DMI, kg/d	18.2	17.2	17.2	0.72	0.56
ADG, kg/d	1.88	2.46	2.54	0.23	0.14
G:F	0.10	0.14	0.15	0.01	0.09
Feed cost/kg gain, $/kg	1.80	1.55	1.54	0.17	0.52
Final, d 0-75					
75 d gain, kg	140.1[f]	127.7[f]	88.8[g]	12.2	0.04
DMI, kg/d	17.6[f]	15.2[g]	14.1[g]	0.48	0.002
ADG, kg/d	1.87[f]	1.70[f,g]	1.18[g]	0.16	0.04
G:F	0.11	0.11	0.08	0.01	0.13
Feed cost/kg gain, $/kg	1.73[g]	1.76[g]	3.78[f]	0.33	0.002
Final BW, kg	740.6[f]	727.5[f,g]	689.8[g]	12.6	0.047
Final BCS (1-9)	7.45	7.48	7.10	0.16	0.19
Yardage costs, $/cow[h]	36.75[f]	36.75[f]	29.15[g]	-	< 0.001
Veterinary medicine costs, $/cow	11.88	12.59	11.99	0.59	0.69

[a]HAY: Hay-based finishing diet consisted of ground mixed hay, cracked corn, alfalfa haylage, finish supplement, soybean meal, MGA® pellets and calcium carbonate.
[b]SILAGE: Silage-based finishing diet consisted of barley silage, cracked barley, ground mixed hay, finish supplement, MGA® pellets and calcium carbonate.
[C]LIMIT: Self-fed finishing diet, offered ad-libitum via self-feeders placed in pens.
[d]Standard error of mean; n = 4 observations per treatment.
[e]*P*-value for protected F test.
[f, g, h]Means with different subscripts differ ($P < 0.05$).

Table 3. Influence of market cow feeding strategy on carcass traits and economics

Item	HAY[a]	SILAGE[b]	LIMIT[c]	SEM[d]	*P*-value[e]
Harvested cows					
HCW, kg	437.3	420.5	416.8	6.85	0.14
Dressing %	54	53	54	0.78	0.88
Lean maturity[f]	448	453	445	19.61	0.96
Skeletal maturity[f]	441	477	446	16.09	0.29
Marbling score[g]	398	390	422	21.81	0.58
12th rib fat thickness, mm	14.2	18.8	15.0	1.27	0.10
Longissimus area, cm	88.4	80.6	77.4	4.52	0.25
Muscling score[h]	2.75	3.25	3.75	0.34	0.18
Fat color[i]	2.75	3.25	2.50	0.26	0.18
Lean color[j]	5.25	6.0	5.50	0.40	0.44
Total cow value, $	1,038.33	999.27	990.32	16.26	0.14
Auctioned cows					
Sale BW, kg	752.9	807.4	720.2	24.02	0.08
Sale price, $/kg	1.31	1.33	1.29	0.01	0.34
Total cow value, $	962.74	1005.51	906.29	41.78	0.29
Breakevens					
Harvested cows, $/kg	1.17[l]	1.19[k,l]	1.24[k]	0.02	0.03
Auctioned cows, $/kg	1.15	1.09	1.23	0.04	0.11
Profit or Loss					
Harvested cows, $/hd	155.34[k]	125.22[k,l]	94.92[l]	13.35	0.03
Auctioned cows, $/hd	122.41	175.61	53.62	40.09	0.15

[a]HAY: Hay-based finishing diet consisted of ground mixed hay, cracked corn, alfalfa haylage, finish supplement, soybean meal, MGA® pellets and calcium carbonate.
[b]SILAGE: Silage-based finishing diet consisted of barley silage, cracked barley, ground mixed hay, finish supplement, MGA® pellets and calcium carbonate.
[c]LIMIT: Self-fed finishing diet, offered ad-libitum via self-feeders placed in pens.
[d]Standard error of mean; n = 4 observations per treatment.
[e]*P*-value for protected F test.
[f]A = 100 to199, B = 200 to 299, C = 300 to 399, D = 400 to 499, and E = 500 to 599.
[g]Slight = 300 to 399 and Small = 400 to 499.
[h]Thin = 1, Average = 3, and Thick = 5.
[i]Pure white = 1, Yellow = 5.
[j]Light red = 1, Cherry red = 4, and Very dark red = 8.
[k, l]Means with different subscripts differ ($P < 0.05$).

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THE EFFECTS OF RE-ENSILING WET DISTILLER'S GRAINS PLUS SOLUBLES WITH CORN SILAGE ON GROWTH PERFORMANCE OF YEARLING BEEF HEIFERS

R. P. Arias[1], L. J. Snyder, [1]R. P. Lemenager[2] and S. L. Lake[3]
Departments of [2]Animal Sciences and [1]Agronomy, Purdue University, West Lafayette, IN
[3]Animal Science Department, University of Wyoming, Laramie, WY

ABSTRACT: The objective of this study was to evaluate the effects of re-ensiling corn silage (CS) with wet distiller's grains plus solubles (WDGS) on growth performance of yearling Angus heifers. Silage used for diets containing re-ensiled CS was ensiled in bags using traditional ensiling parameters, allowed time for proper fermentation (30-d); bags were opened and then re-ensiled with WDGS. Ninety-six heifers (296 ± 28.9 kg BW; 5.42 ± 0.36 BCS) were blocked by weight and randomly assigned to one of four dietary treatments formulated to meet or exceed NRC (1996) nutrient requirements: 1) CS plus soybean meal (**CON**), 2) CS re-ensiled with WDGS (**RE-EN**; 3:1 CS:WDGS DM basis), 3) CS mixed with WDGS at feeding (**CS+WDG**) and, 4) CS mixed with dry distiller's grains plus solubles (DDGS) at feeding (**CS+DDG**). Rations were limit-fed to obtain 0.82 kg/d ADG. Heifer BW and BCS were recorded on two consecutive days at the beginning and the end of the trial. DMI was adjusted as needed based on BW every three weeks. Heifers fed CS+WDG had decreased DMI ($P < 0.01$) when compared to all other treatments, therefore; performance parameters were analyzed using DMI as a covariate. Heifer ADG was greatest ($P < 0.01$) on RE-EN compared with CS+DDG diet with CS+WDG intermediate. CON fed heifers had lower ADG compared to all other treatments. Heifer G:F was greater ($P < 0.01$) for those consuming RE-EN compared with all other diets, and heifers fed CS+DDG had greater G:F than CON fed heifers, with CS+WDG being intermediate. There were no differences in final BCS ($P = 0.21$) or change in BCS ($P = 0.43$) due to treatments. These data suggest that re-ensiling CS with WDGS produces performance in growing heifer rations that is equivalent, or superior, to adding soybean meal, DDGS or WDGS at the mixer. Moreover, the re-ensiling process allows small production units the opportunity to capitalize on the purchase, storage and feeding of WDGS without the concern of spoilage or lost animal performance.

Key words: beef heifers, WDGS, re-ensiling

Introduction

The exponential growth in the ethanol industry has increased demand for cereal grains and has resulted in higher feed costs for livestock producers, which has placed small and medium-sized cattle operations at a competitive disadvantage. Fortunately, ethanol co-products, either wet (WDGS) or dry distiller grains with solubles (DDGS), provide an energy and protein dense, highly fermentable fiber source that works well in cattle diets. Dry distiller's grains plus solubles have resulted in an excellent alternative to corn in terms of feed value and storage properties; however, the energy used to dry the wet product elevates its cost of production, and consequently its price, to values close or equal to corn itself. As long as transportation costs are not exuberant, WDGS may provide a better value due to its lower processing cost. Unfortunately, utilization of WDGS for small to mid-sized operations is not always feasible since this co-product is rapidly perishable (3 – 7 days depending on temperature and relative humidity; Plain, 2006), and storage in bags or silos is not recommended due to compaction, bulk density, and bridging properties of the feed (Buckmaster et al, 2008). Nevertheless, successful storage and co-ensiling of WDGS with corn silage has been reported (Garcia and Kalscheur, 2004; Arias et al., 2008; Buckmaster et al., 2008). The challenge for producers is now to overlap the time when corn is harvested for silage making, and the time when WDGS are available in the market at a reasonable price. In general, when corn is ready for ensiling, producers will dedicate all labor to finish this task in a short period of time, leaving little or no time for WDGS hauling and co-ensiling practices. For this reason, re-ensiling WDGS with corn silage is proposed in this study as an alternative feed conservation practice. For the purpose of this study, we will define **re-ensiling** as the utilization of corn silage that was previously ensiled using traditional ensiling parameters, allowed for proper fermentation, opened and re-ensiled with WDGS. This way, ensiling corn does not have to be linked to WDGS availability; moreover, the re-ensiling can be done at anytime. We hypothesized in this study that re-ensiled corn silage plus WDGS will result in equivalent nutritional value and animal performance, as that obtained by mixing soybean meal WDGS or DDGS at feeding, with corn silage. Therefore, the objective of this study was to evaluate the effects of re-ensiling corn silage with WDGS on growth performance of yearling Angus heifers.

Materials and Methods

Animals. All procedures for the following experiment were approved by the Purdue University Animal Care and Use Committee. Ninety-six yearling Angus-cross heifers (296 ± 28.9 kg of initial BW; 5.42 ± 0.36 of initial BCS) were weighed and body condition scored on two consecutive days at the beginning and the end of the trial. Weighs were recorded every three weeks to monitor performance and to adjust DMI. Heifers were blocked by BW and BCS in a completely randomized design with four heifers per pen and assigned to one of four dietary

treatments (6 pens/treatment). The study length was 73 days.

Diets. All diets were formulated to meet or exceed NRC (1996) nutritional requirements and were designed to be isocaloric and isonitrogenous. Rations were limit-fed to obtain an ADG of 0.82 kg/day. Specific composition information about the diets is summarized in Table 1 and Table 2. Heifers were randomly assigned to 1 of 4 dietary treatments: 1) a control diet (CON) consisting of corn silage and soybean meal, and was used to compare the distillers grains based diets to a traditional ration, 2) a re-ensiled diet consisting of corn silage and WDGS. Corn silage used for this diet was originally ensiled for 30 days, the bag of silage was opened and re-ensiled with WDGS for 30 more days in Ag-Bags® (RE-EN), 3) corn silage with WDGS mixed at time of feeding (CS+WDG), and 4) corn silage with DDGS mixed at time of feeding (CS+DGS). All distillers grains based diets are equivalent in terms of corn by-product percent inclusion on a DM basis.

Statistical analysis. Heifer performance data was analyzed using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC) for a completely randomized design with pen as the experimental unit. Significant means ($P < 0.05$) were separated using the LSD method. DMI was introduced in the model as a covariate to analyze performance variables. It should be noted that once the covariate (DMI) is included in the model to adjust ADG, BW change, final BW, BCS change and final BCS, the reported values for these variables become predicted values.

Results and Discussion

Descriptive statistics are presented in Table 3. By design, there were no differences in initial BW or initial BCS across treatments. Heifers fed CS+WDG had decreased ($P < 0.01$) DMI when compared to all other treatments, therefore; change in BW, final BW, ADG, G:F, as well as change in BCS and final BCS were analyzed using DMI as a covariate. Average daily gain was greatest ($P < 0.01$) for heifers fed the RE-EN diet compared with heifers fed the CS+DDG diet. Heifers fed the CS+WDG were intermediate in ADG to both the RE-EN and CS+DDG fed heifers; However, CON fed heifers were the lowest compared to all other treatments. Heifer G:F was greatest ($P < 0.01$) for those consuming RE-EN compared with all other diets,. Heifers fed CS+DDG had greater G:F than CON fed heifers, with CS+WDG being intermediate. Similar performance results were reported by Klopfenstein et al. (2007), who showed that WDGS produced higher ADG and G:F when compared to cattle fed corn-based diets without distiller's grains. Additionally, Ham et al (1994) reported comparable gains but greater feed efficiency and lower DMI in cattle fed WDGS compared to cattle fed DDGS. Arias et al. (2008) also reported higher efficiency in pregnant heifers fed co-ensiled corn silage plus WDGS (similar to RE-EN treatment) when compared to corn silage plus soybean meal and corn silage plus distiller's grains (either dry or wet) when mixed at feeding. Final BW was highest ($P < 0.01$) for heifers fed the CS+WDG diet compared to all other diets. RE-EN and CS+DDG diets resulted in heifers with intermediate final BW and heifers fed CON the lowest. However, BW gain was highest for heifers fed the RE-EN diet, intermediate for the CS+WDG diet, but not different than CS+DDG and lowest for CON. Weight gains were also similar to those observed by Arias et al. (2008) where the co-ensiled corn with WDGS resulted in greater overall weight gain when compared to CON and CS+DGS equivalent treatments on pregnant heifers. There were no differences in final BCS ($P = 0.17$) or change in BCS ($P = 0.37$) due to treatments. It is important to point out that ADG and change in BW were higher in the RE-EN treatment (even though it did not differ from the CS+WDG treatment), and at the same time final BW was highest for the CS+WDG diet and significantly different from RE-EN fed heifers. This incongruence can be explained by the fact that once the covariate (DMI) was included in the model to adjust ADG, BW change, final BW, BCS change and final BCS, the reported values for these variables become predicted values.

Implications

These data suggests that re-ensiling corn silage with WDGS produces performance in growing heifer rations that is at least equivalent, if not superior to adding soybean meal, DDGS or WDGS at the mixer. Moreover, the re-ensiling process allows small and mid-sized production units the opportunity to capitalize on the purchase, storage and feeding of WDGS without the concern of spoilage or lost animal performance.

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Table 1. Ingredient composition of heifer diets

Ingredient	Diets[1] (% of DM)			
	CON	**RE-EN**	**CS+ WDG**	**CS+ DDG**
Corn Silage[2]	66.0	—	64.6	64.6
Cracked corn	17.2	—	—	—
Soybean meal	13.5	—	—	—
Re-ensiled material[3]	—	96.7	—	—
DDGS[4]	—	—	—	32.1
WDGS[5]	—	—	32.1	—
Mineral premix[6]	3.3	3.3	3.3	3.3

[1]CON = control (corn silage with soybean meal), RE-EN = re-ensiled with wet distiller's grains plus solubles (3;1 DM basis), CS+WDG = corn silage plus WDGS added at mixing, CS+DDG = corn silage plus DDGS added at mixing.
[2] Corn silage: 40% DM, 7.9% CP, 1.3 Mcal/kg of Neg (DM basis).
[3] Re-ensiled corn silage with WDGS (3:1 DM basis).
[4] DDGS = Dry distillers grains plus solubles.
[5] WDGS = Wet distillers grains plus solubles.
[6] 70% $CaCo_3$, 11.5% inorganic mix, 18.5% NaCl.

Table 2. Formulated nutrient content of heifer diets (DM basis)

Ingredient	Diets[1,2]			
	CON	**RE-EN**	**CS+ WDG**	**CS+ DDG**
NEg, Mcal/Kg	1.15	1.15	1.15	1.15
CP, %	14.5	14.5	14.5	14.5
Crude Fiber, %	16.5	28.3	28.3	28.3
TDN	73.4	73.5	73.5	73.5
DM, %	54.0	38.0	46.0	54.0

[1]CON = control (corn silage with soybean meal), RE-EN = re-ensiled corn silage with wet distiller's grains plus solubles (3:1 DM basis), CS+WDG = corn silage plus WDGS added at mixing, CS+DDG = corn silage plus DDGS added at mixing.
[2]Dietary energy and protein were formulated using tabular values (NRC, 1982).

Table 3. Effect of treatments on performance of yearling Angus heifers

Item	Treatments[1,2]					
	CON	**RE-EN**	**CS+WDG**	**CS+DDG**	***SEM*[3]**	***P***
DMI, kg/day	6.41[a]	6.44[a]	6.07[b]	6.38[a]	0.27	<0.001
Initial BW, kg	296	297	297	296	6.73	0.962
Initial BCS	5.41	5.41	5.43	5.41	0.08	0.999
ADG, kg[4]	1.09[c]	1.26[a]	1.25[ab]	1.18[b]	0.04	0.001
G:F[4]	0.172[c]	0.205[a]	0.185[bc]	0.188[b]	0.01	0.001
Final BW, kg[4]	375[c]	387[b]	401[a]	381[b]	15.08	<0.001
Final BCS[4]	5.34	5.44	5.47	5.48	0.05	0.166
Change in BW, kg[4]	83.7[c]	97.36[a]	96.2[ab]	90.7[b]	2.77	0.001
Change in BCS[4]	-0.07	0.05	-0.11	0.05	0.08	0.368

[1] CON = control (corn silage with soybean meal), RE-EN = re-ensiled corn silage with wet distiller's grains plus solubles 3:1 (DM basis), CS+WDG = corn silage plus WDGS added at mixing, CS+DDG = corn silage plus DDGS added at mixing.
[2] Means within a row lacking a common superscript differ ($P \leq 0.05$).
[3] The greatest SEM is presented (n = 6 pens/treatment).
[4] Mean predictions, adjusted by covariate using DMI.

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MICROHISTOLOGICAL CHARACTERIZATION OF THE BOTANICAL COMPOSITION OF DIETS GRAZED BY BEEF COWS IN THE KANSAS FLINT HILLS DURING WINTER

G. J. Eckerle[†], K. C. Olson[†], J. R. Jaeger[†], W.H. Fick[††], L. A. Pacheco[†], and K. Walburger[‡]
[†]Department of Animal Science, Kansas State University, Manhattan, Kansas, USA, 66502
[††]Department of Agronomy, Kansas State University, Manhattan, Kansas, USA, 66502
[‡]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada

ABSTRACT: A study was conducted on native tallgrass range in the Kansas Flint Hills to establish the validity of using microhistological analysis of cattle feces to quantify the botanical composition of mature cow diets grazed during winter. Standard microscope slides were prepared of cross-sectional leaf and stem particles from 10 predominant range plants (Andropogon *geradi* (AG); Schizachyrium *scoparium*; Bouteloua *curtipendula*; Bouteloua *gracilis* (BG); Panicum *virgatum*, Sorghastrum *nutans* (SN), Amorpha *canescens* (AC), Symphyotricum *ericoides*, Liatris *punctata*, and Dalea *purpurea* (DP)). Mature beef cows (n = 10) were allowed to graze a single native tallgrass pasture for 30 d. Subsequently, 1000 g of wet fecal material was collected from each cow. Fecal material was dried and ground to pass a 1 mm screen; subsamples were affixed to slides (5 slides / cow) and compared to standards. Each slide was divided into twenty fields. Range-plant particulate counts were analyzed by animal; plant prevalence in fecal material was assumed to be equivalent to diet composition on a DM basis. Diets were composed of 66% grass and 34% forbs. Predominant grass species included AG (9%), BG (16%), and SN (13%). Predominant forbs were AC (8%), and DP (14%). This subgroup of plants represented 54% of the diets selected by cows in our study. Nine percent of grass-plant fragments could not be identified with any of the grasses for which standard were prepared: moreover, 4% of forb fragments could not be identified with any of the forbs for which standards were prepared. Unknown plant fragments composed 6% of all plant fragments in our slide fields. In conclusion, methods for measuring diet composition of herbivores from microhistological examination of fecal samples were successfully adapted to cattle grazing native range in the Kansas Flint Hills during winter. Results of this study may allow for the management of rangeland for the propagation of forages that are more heavily consumed by grazing ungulates.
Keywords: botanical composition, cattle, grazing, microhistology,

Introduction

Microhistological analysis has been widely used for determining diet composition and habitat usage by wild and domestic herbivores (Holechek et al., 1982). Validation of the methods used to analyze botanical composition of grazed forages from fecal material has been conducted by Sparks and Malechek (1968) and Holechek and Gross (1982). Variation was noted in fecal recovery of certain forages based upon differences between animal species (Leslie et al., 1983). Previous studies have concluded that fecal microhistology is an effective method for determining dietary composition when there is an inability to monitor grazing behavior or when collection of masticate samples in not practical (Anthony and Smith, 1974; Holechek, 1982; Lewis, 1994) . Comparisons between fecal and masticate sample analysis have demonstrated that fecal analysis is an acceptable method for determining dietary composition of ungulate animals (Anthony and Smith, 1974; Wydeven et al., 1982; Lewis, 1994; Chapuis et al., 2001).

There have been questions about whether plant phenology can mask microhistological differences between forage species. Holechek et al. (1982) examined the effects of plant growth stage on microhistological characteristics and concluded that plant maturity had little influence on the ability of observers to differentiate between forage species.

Primarily, fecal samples have been used to estimate the botanical composition of diets selected by grazing ruminants. In our study, the prevalence of 10 predominant grasses and forbs native to the Kansas Flint Hills were examined in the diets of grazing, mature beef cows. Our objective was to establish the validity of using microhistological analysis of cattle feces to quantify the botanical composition of diets grazed by mature beef cows during winter.

Materials and Methods

All procedures used in the care, handling, and sampling of animals in our study were approved by the Kansas State University Institutional Animal Care and Use Committee.

Sample Collection. Mature, non-pregnant beef cows (n=10; average initial weight = 530 ± 26 kg)

were maintained on a single, dormant, native Tallgrass pasture at the Kansas State University Commercial Cow-Calf Unit. Approximately 95% of above-ground biomass on these pastures was composed of the following forage species: Big Bluestem (Andropogon *geradii*; **AG**), Little Bluestem (Schizachyrium *scoparium*; **SS**), Sideoats Gramma (Bouteloua *curtipendula*; **BC**), Blue Gramma (Bouteloua *gracilis*; **BG**), Switch Grass (Panicum *virgatum*; **PV**), Indian Grass (Sorghastrum *nutans*; **SN**), Lead Plant (Amorpha *canescensi;* **AC**), Heath Aster (Symphyotricum *ericoides*; **SE**), Dotted Gayfeather (Liatris *punctata*; **LP**), and Purple Prairie Clover (Dalea *purpurea*; **DP;** Haddock, 2005). Cattle were allowed to adapt to the pasture for 30 d. On d 31 of the experiment, animals were temporarily moved to a corral. Approximately 1000 g of wet fecal material was collected from each cow using the rectal grab technique. Wet fecal samples were sealed in plastic containers. Samples were subsequently hand-mixed and a 40-g subsample was removed for analysis.

Standard Preparation. Standard slides of each forage type were prepared approximately 14 d prior to the study. Each standard was derived from a pure sample of each forage type. Slides were prepared using the methods described by Holechek (1982). Training of observers was required to obtain an acceptable accuracy of identification of plant fragments. Training methods were described by Holecheck and Gross (1982). Briefly, observers viewed standard slides to become familiar with individual differences between forage species. Observers then viewed slides at random until they could identify each species based on anatomical differences. Plant fragments that appeared in cattle diets that were not one of the 10 predominant range plants for which standards were prepared were classified as either an unknown grass or an unknown forb. In our experiment, the proportion of all grass fragments that were classified in this manner was 9%; moreover, the proportion of all forb fragments that were classified as unknown was 4%.

Sample Preparation. Samples were prepared using methods described by Holechek (1982). Fecal samples were soaked in a 50% (v/v) ethanol solution overnight. After soaking, samples were homogenized in a blender, rinsed with de-ionized water, and filtered through a No. 200 US-standard testing sieve to remove contaminants. Excess water was drained and the samples were placed in a drying oven at 55°C for 96 h. Finally, samples were ground (#4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) through a 1-mm screen and stored in plastic containers for subsequent slide preparation (Bennett et al., 1993).

Slide Preparation. Slides also were prepared using methods described by Holechek (1982). Dried fecal samples (1.5 – 2 g) were placed in beakers and soaked in distilled water for 1 h. Sodium hydroxide (0.05 M) was then added to each beaker and samples were soaked for an additional 20 min to destroy plant pigments. Sodium hydroxide was removed by washing the samples with de-ionized water over a No. 200 US-standard testing sieve. After NaOH was removed, the samples were placed in a blender with approximately 25 ml of distilled water and homogenized for 1 min.

One to 2 drops of Hertwig's solution was placed on each microscope slide immediately before samples were mounted to allow samples to be positioned on the slide more easily. Once samples were positioned on the slides, they were held over a propane flame until the Hertwig's solution evaporated. Hoyer's solution was added to slides and they were again held over a propane flame in order to fix the samples in place. Prepared slides were dried in a 55°C oven for 96 h before viewing.

Dried slides were read on a compound microscope at 10x magnification. The microscope was equipped with a digital camera; each slide field was photographed for comparison with standard slides. Twenty fields per slide were selected randomly from the entire slide view and were used to measure the frequency with which plant fragments appeared. Plant fragment prevalence in slide fields was assumed to be equivalent to prevalence in fecal samples and in grazed diets on a DM basis.

Statistics. All data were analyzed as a completely random design using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Class variables included animal, slide, and field within slide. The model included terms for forage species, animal, slide, and field. Type-3 error rates were used to report differences between forage species. A simple t-test was used to test for differences between means. Means were separated using the method of Least Significant Difference and reported with a pooled standard error. Means were considered to be different when $P \leq 0.05$.

Results and Discussion

Although the cattle used in our study were of the same class (i.e., mature cows) and age (i.e., 4 y), there were differences ($P < 0.01$) between animals in the proportions of various range plants that were grazed. Table 1 summarizes mean values and SEM for 10 predominant grass and forb species in the diets of beef cows in our trial. Overall, grasses composed 65.74% of the diet: 8.91% was AG, 8.07% was SS, 15.06% was BC, 8.88% was BG, 8.14% was PV, and 12.95% was SN. Nine percent of grass-plant fragments could not be identified with 1 of the 6 grasses for which standards were prepared. A preference for BC and SN was apparent within this particular group of cattle.

A significant proportion of cow diets (34.26%) was composed of forbs, in spite of the fact that the experiment was conducted on dormant winter range and most forbs were difficult to locate visually. Forb composition in the diets of grazing cows was 7.89% AC, 7.00% SE, 4.18% LP, and 13.26% DP; 4% of forb fragments could not be identified with the 4 forb species for which standards were prepared. Altogether, plant fragments that could not be assigned a positive species identification composed 6% of the diet. We interpreted these data to indicate that a relatively minor proportion of all plant fragments in fecal samples could not be classified according to plant species.

Prior research indicated that there were differences in botanical composition of diets when estimates were made from fecal samples compared with masticate samples. Differences were due in part to variations in plant digestibility and time of plant consumption (Anthony and Smith, 1974; Wydeven, 1982; Lewis, 1994). These studies indicated that fecal analysis tended to overestimate dietary consumption of shrubs and grasses compared to analysis of ruminal contents (Lewis, 1994; Chapuis et al., 2001). In contrast, Lewis (1994) noted that microhistological analysis of fecal matter tended to underestimate the forb component of the diet compared to microhistological analysis of ruminal contents.

Carrière (2002) noted that it was occasionally difficult for researchers using the microhistological technique to differentiate between closely-related plant species. In our study, this was true of AG and SS. In instances where technicians identified individual plant fragments as either AG or SS but were unable to discern any species-specific characteristics, the observations were classified as either an unknown grass or an unknown forb.

Questions have arisen regarding the proper sample size when assessing treatment differences in botanical composition of grazed diets using the fecal microhistological technique. Anthony and Smith (1974) calculated that fecal microhistological analysis of diet composition required a minimum of 15 animals per treatment. Under the conditions of our study, a minimum sample size of 13 mature cows (range = 3.1 cows for DG to 12.4 cows for BB) was calculated to be necessary to detect a difference in mean species preference of 2% with 95% confidence, across the 10 predominant range plants in the Tallgrass Prairie region (Sokal and Rohlf, 1969).

Results from our study were interpreted to indicate that the methods derived and verified by Holechek et al. (1982), Sparks et al. (1968), and Bennett et al. (1993) were viable means for estimating the botanical composition of mature cow diets grazed during the winter in the Tallgrass Prairie of central Kansas.

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Table 1. Botanical composition of diets selected by beef cows grazing Kansas Flint Hills range during winter.

Species	Botanical Composition (% of diet DM)	Minimum	Maximum	SE	CV (%)
Grasses					
Andropogon *geradii*	8.91	0	10	0.141	7.01
Schizachyrium *scoparium*	8.07	0	6	0.127	6.97
Bouteloua *curtipendula*	15.06	0	10	0.128	3.76
Bouteloua *gracilis*	8.88	0	8	0.107	5.34
Panicum *virgatum*	8.14	0	9	0.129	7.24
Sorghastrum *nutans*	12.95	0	10	0.135	8.54
Unidentified grasses	9.00	0	20	0.161	1.46
Forbs					
Amorpha *canescensi*	7.89	0	10	0.111	6.04
Symphyotricum *ericoides*	7.00	0	8	0.095	3.25
Liatris *punctata*	4.18	0	5	0.085	9.01
Dalea *purpurea*	13.26	0	10	0.124	4.14
Unidentified forbs	4.00	0	20	0.144	1.81

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EFFECTS OF SUPPLEMENTAL ENERGY ON POST-RUMINAL UTILIZATION OF TRYPTOPHAN BY GROWING LAMBS

S. A. Kuykendall, L. E. Camacho, L. Chen, and C. A. Löest
New Mexico State University, Las Cruces, NM

ABSTRACT: Inadequate dietary supply of Trp limited N retention of growing lambs fed a diet low in ruminally undegradable protein. Therefore, increasing Trp utilization may improve N retention. The objective was to evaluate the effects of supplemental energy on post-ruminal utilization of Trp in growing lambs. Eight ruminally cannulated wethers (49.5 ± 0.64 kg initial BW) housed in metabolism crates were limit-fed (813 g DM/d) twice daily a soybean hull-based diet low in ruminally undegradable protein. Treatments (2 × 2 factorial) were 2 amounts of energy (0 vs 0.4 Mcal GE/d) and 2 amounts of L-Trp (0 vs 1 g/d). Energy was supplied via continuous ruminal infusions of acetate (35 g/d) and propionate (10 g/d) and continuous abomasal infusions of dextrose (60 g/d). The L-Trp was supplied with continuous abomasal infusions of an L-AA solution (500 mL/d that supplied 2.0, 8.2, 4.0, 2.0, 3.4, 7.0, 3.3, 3.7, 6.6, 6.0, and 11 g/d Ile, Leu, Val, Met, His, Lys, Thr, Phe, Arg, Gly, and Glu). The experiment was a replicated 4 × 4 Latin square with 11-d periods, allowing 4 d for resting, 3 d for adaptation to treatments, and 4 d for collection of feces and urine. Blood samples were collected 3 h after feeding on d 11. No Trp × energy interactions ($P \geq 0.13$) were observed for OM, NDF, and N intake, feces, and digestibility, N retention, serum urea N, glucose, insulin, IGF-I, and plasma AA. Total N intake (dietary + infused) increased ($P < 0.01$) in response to Trp infusion, but N digestibility and N retention were not affected ($P \geq 0.31$). Plasma concentrations of Ile, Trp, Asn, and Pro increased ($P \leq 0.05$) with Trp infusion. Energy infusion decreased ($P < 0.02$) urinary N excretion, and increased ($P < 0.01$) N retention. Infusion of energy decreased ($P \leq 0.05$) serum urea N and plasma Ile, Ala, Glu, and Pro concentrations, and increased ($P \leq 0.02$) plasma Gly and Ser concentrations. These results indicated that Trp was not a limiting AA for growing lambs fed a soybean hull-based diet, but energy increased the efficiency with which lambs utilized metabolizable AA for growth.

KEYWORDS: energy, tryptophan, sheep

INTRODUCTION

When Trp replaced isonitrogenous amounts of urea in a semi-purified diet, N retention of growing lambs improved (McLaren et al., 1965). Because the Trp source was not protected from ruminal degradation, it cannot be determined whether this response was due to changes in rumen microbial fermentation, changes in metabolizable AA supply, or both. However, Nolte et al. (2008) demonstrated that Trp was one of several limiting AA in the metabolizable supply of growing lambs when fed a diet from which the major supply of MP was microbial protein. Additionally, the efficiency with which post-ruminal Trp supply was used for protein deposition in growing lambs was only 13.4% (Nolte et al., 2008).

The efficiency with which AA are utilized for protein synthesis is, in part, affected by the need for those AA in other metabolic process, such as glucogenic precursors. Schroeder et al. (2006) demonstrated that supplemental energy increased the efficiency of AA use in growing steers. Also, Kuykendall et al. (2008) demonstrated that supplementation of ruminally unavailable energy increased N retention of growing lambs even when Met was limiting. Because these studies suggest that energy supply affects AA utilization, we hypothesized that additional energy supply might increase Trp utilization for growth. The objective of this study was to evaluate the effects of supplemental energy on post-ruminal utilization of Trp in growing lambs fed a soybean hull-based diet.

MATERIALS AND METHODS

Animals, Design, and Diet

New Mexico State University's Institutional Animal Care and Use Committee approved animal handling and experimental procedures. Eight ruminally cannulated wether lambs (49.5 ± 0.64 kg initial BW) were used in a replicated 4 × 4 Latin square. Lambs were housed individually in metabolism crates under continuous light and evaporative cooling. Periods were 11 d, allowing 4 d for resting, 3 d for adaptation to treatments, and 4 d for collection of feces and urine.

Lambs had free access to water and were limit-fed (813 g DM/d) a soybean hull-based diet (Table 1) every 12 h (700 and 1900) in equal portions. The diet was formulated to be low in ruminally undegrable protein, so that the predominant source of metabolizable AA was from microbial protein. Lambs were adapted to the diet for 14 d before the start of experimental periods.

Treatments

Treatments were arranged as a 2 × 2 factorial with 2 amounts of supplemental energy (0 vs 0.4 Mcal GE/d) and 2 amounts of abomasally infused L-Trp (0 vs 1 g/d). The supplemental energy was supplied via continuous ruminal infusions of acetate (35 g/d) and propionate (10 g/d) and abomasal infusions of dextrose (60 g/d). The L-Trp was supplied with continuous abomasal infusions of an L-AA solution. The L-AA solution (500 mL/d) supplied 2.0, 8.2, 4.0, 2.0, 3.4, 7.0, 3.3, 3.7, 6.6, 6.0, and 11 g/d of L-Ile, L-

Leu, L-Val, L-Met, L-His, L-Lys, L-Thr, L-Phe, L-Arg, Gly, and L-Glu to ensure that these AA did not limit N retention. The amounts of L-AA infused were calculated based on the differences between the metabolizable supply of AA from the soybean hull-based diet and the AA requirements of growing lambs (Nolte et al., 2008). Treatments were delivered using flexible tubing and a Manostat cassette pump (Barnant Co. Barrington, IL.). For abomasal infusions, the flexible tubing was passed through the rumen cannula and reticulo-omasal orifice.

Table 1. Diet composition

Item	% of DM
Ingredient	
Soybean hulls	79.6
Alfalfa hay	15.0
Cane molasses	3.5
Mineral/Vitamin premix[1]	0.80
Sodium bicarbonate	0.50
Urea	0.35
Salt	0.20
Elemental sulfur	0.05
Nutrient	
NDF	56.3
CP	14.8
RDP[2]	11.7

[1]Composition: Ca (14 to 17%), P (≥ 11%), NaCl (11 to 13%), Mg (≥ 0.5%), K (≥ 0.1%), Cu (5 to 7 mg/kg), Se (≥ 15 mg/kg), Zn (≥ 1980 mg/kg), Vit A (660 KIU/kg), Vit D (165 KIU/kg), Vit E (1.32 KIU/kg).
[2]Ruminally degradable protein, calculated using NRC (2000) table values.

Collections

Diet samples, orts (if any), total feces, and total urine were collected on d 8 through 11 of each period. Feces were collected daily in steel pans, and urine was collected in 2.5 L glass bottles containing 50 mL of 6 *N* HCl to minimize NH_3 loss. Total fecal and urinary output was recorded, and the feces and a 5% representative sample of urine were frozen. Before analysis, samples were thawed and composited by period for each lamb.

Blood samples were collected 3 h after the morning feeding on d 11 of each period. Blood was collected from the jugular vein into vacuum tubes with (Monoject, Tyco Healthcare Group LP, Mansfield, MA) and without (Corvac, Tyco Healthcare Group LP, Mansfield, MA) sodium heparin. Samples for plasma were immediately chilled on ice and samples for serum were allowed to coagulate at room temperature for 30 min. All blood samples were centrifuged at 1,300 × g for 15 min at 4°C, transferred to 7 mL plastic vials, and frozen (-20°C) for later analysis.

Sample Analysis

Samples of diet, orts, and feces were dried in a forced-air oven (Blue M Electric Company, Blue Island, IL) for 72 h at 55°C, allowed to air-equilibrate for 24 h, weighed to determine moisture loss, and then ground to pass a 2-mm screen (Wiley mill, Thomas Scientific, Swedesboro, NJ). Ground dietary and fecal samples were analyzed for DM (105°C for 24 h) in a convection oven (Precision Scientific, Chicago, IL), OM (500°C for 8 h) in a muffle furnace (Thermolyne Corp., Dubuque, IA), and NDF (ANKOM Technology Corp., Fairport, NY). Samples of diet, orts, feces, and urine were analyzed for N via total combustion (LECO Corp., St. Joseph, MI).

Serum concentrations of urea N and glucose were analyzed colorimetrically (Microplate Reader, Biotek Instruments Inc., Winooski, VT) using commercially available reagents (Infinity TR12421 and TR15321; Thermo Scientific, Waltham, MA). Serum insulin (Reimers et al., 1982) and IGF-I (Berrie et al., 1995) concentrations were determined by solid-phase RIA. Plasma AA concentrations were analyzed by GLC (Varian CP-3800 GC, Varian Inc. Walnut Creek, CA) using a commercially available kit (EZ:FAAST KGO-7165; Phenomenex, Torrance, CA) as described by Waggoner et al. (2009).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included effects of period, energy, Trp, and energy × Trp, with lamb as the random effect. Data are presented as least square means, and differenced were considered significant when $P \leq 0.05$.

RESULTS

Treatment Interactions

Energy × Trp interactions ($P \geq 0.23$) were not significant for OM, NDF, and N intake, fecal OM, NDF, and N excretion, OM, NDF, and N digestibility, and N retention. Similarly, serum metabolites and plasma AA did not exhibit energy × Trp interactions ($P \geq 0.13$). Therefore, means for the effects of Trp and energy are presented separately.

Effects of Trp

Effects of Trp on nutrient intake, digestibility, and N retention are presented in Table 2, and effects of Trp on serum metabolites and plasma AA are in Table 3. By design, abomasal infusions of Trp increased ($P < 0.01$) total N intake (dietary + infusions). However, Trp infusion did not affect ($P \geq 0.27$) OM and NDF intake, fecal OM, NDF and N excretion, OM, NDF and N digestibility, urinary N excretion, and N retention. Infusion of Trp also did not affect ($P \geq 0.10$) serum urea N, glucose, insulin, and IGF-I, but increased ($P \leq 0.05$) plasma concentrations of Ile, Trp, Asn, and Pro.

Effects of Energy

Effects of energy on nutrient intake, digestibility, and N retention are presented in Table 4, and serum metabolites and plasma AA are in Table 5. Energy infusion did not

affect ($P \geq 0.40$) OM, NDF and N intake, feces, and digestibility, but decreased ($P < 0.01$) urinary N excretion and increased ($P < 0.01$) N retention. Serum IGF-I, insulin, and glucose were not affected ($P \geq 0.15$), but serum urea N decreased ($P = 0.02$) with the infusion of energy. Infusion of energy also decreased ($P \leq 0.05$) plasma Ile, Ala, Glu, and Pro concentrations, and increased ($P \leq 0.02$) plasma Gly and Ser concentrations.

DISCUSSION

Supply of Trp

Abomasal infusion of L-Trp was effective at supplying absorbable Trp, as evidenced by increases in plasma Trp concentrations in lambs. However, N retention was not improved by the additional supply of absorbable Trp, which indicated that the metabolizable supply of Trp from the basal diet was not limiting. These findings are in contrast to that of Nolte et al. (2008), who reported that N retention of growing lambs fed a soybean hull-based diet was limited by inadequate Trp. The lambs used in our study were heavier than those used by Nolte et al. (2008), and may have had a slower growth rate with potentially lower Trp requirements. Also, we infused less energy to lambs than Nolte et al. (2008) which could also have affected Trp requirements. Our findings are consistent with Storm and Ørskov (1984), who reported that Trp did not limit N retention of lambs maintained by intragastric nutrition. In general, lambs maintained by intragastric nutrition also have slow rates of growth and consequently low AA requirements.

Supply of Energy

Infusion of energy improved N retention and decreased several plasma AA concentrations, indicating that energy limited protein deposition in growing lambs. These findings are consistent with other studies in sheep (Kuykendall et al., 2008) and cattle (Schroeder et al., 2006). A decrease in serum urea N is indicative of decreased AA deamination, possibly because the supply of additional energy decreased the use of AA as glucogenic precursors. This is supported by increases in plasma Gly and Ser concentrations, possibly because of decreased conversion of their carbon skeletons to pyruvate in the liver for the production of glucose. Decreases in serum urea N, and increases in plasma Gly and Ser in response to energy infusion was also reported in sheep (Kuykendall et al., 2008) and cattle (Schroeder et al., 2006).

Energy for Trp Utilization

We hypothesized that additional energy supply might increase Trp utilization for growth of lambs. A greater N retention response to abomasally infused Trp when energy is supplemented compared to no supplemental energy would imply improved utilization of the Trp for growth. However, abomasal infusion of Trp did not increase N retention regardless of energy supply, and therefore was not a limiting AA. Consequently, the effects of energy on the utilization of Trp could not be evaluated. Nonetheless, an increase in N retention and a decrease in plasma AA in response to energy infusion suggest that energy supply may alter post-absorptive AA metabolism as a whole. Similar results have been reported in growing cattle (Schroeder et al., 2006) and sheep (Kuykendall et al., 2006; 2008).

Conclusions

The result of this experiment demonstrated that Trp was not a limiting AA for growing lambs fed a soybean hull-based diet, and that infusion of energy affected post-absorptive AA utilization in growing lambs. Therefore, energy supply may need to be considered when determining AA requirements for sheep.

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Table 2. Effects of abomasal Trp infusion on nutrient intake, digestibility and N retention of growing lambs

	Trp, g/d[1]			*P*-
Item	0	1	SEM[2]	value
Intake, g/d				
OM	734	734	0.20	0.40
NDF	458	458	0.09	0.40
N[3]	28.5	28.6	0.005	<0.01
Fecal, g/d				
OM	113	118	5.69	0.34
NDF	67.4	71.2	3.51	0.29
N	5.10	5.40	0.26	0.27
Urinary, g/d				
N	12.1	11.7	0.64	0.53
Digestibility, %				
OM	84.7	83.9	0.77	0.34
NDF	85.3	84.5	0.77	0.29
N	82.1	81.1	0.89	0.31
Retention				
N, g/d	11.4	11.6	0.77	0.76
N, %	39.8	40.4	2.70	0.81

[1]Continuous abomasal infusions of L-Trp (0 vs 1 g/d) dissolved in an essential AA solution.
[2]Standard error of the mean (n = 15).
[3]Dietary intake + abomasally infused.

Table 3. Effects of abomasal Trp infusion on serum metabolites and plasma AA in growing lambs

	Trp, g/d[1]			*P*-
Item	0	1	SEM[2]	value
Serum				
Urea N, mg/dL	11.9	11.1	0.60	0.10
Glucose, mg/dL	72.0	76.9	2.20	0.11
Insulin, ng/mL	0.69	0.83	0.10	0.15
IGF-I, ng/mL	109	117	9.61	0.22
Plasma AA, μ*M*				
Ala	121	126	5.68	0.48
Asn	26.3	30.5	1.59	0.01
Asp	3.67	4.12	0.23	0.17
Cys	1.07	0.95	0.06	0.14
Gln	292	313	12.5	0.09
Glu	65.6	70.1	3.86	0.41
Gly	548	498	36.8	0.12
His	67.5	65.2	4.04	0.63
Ile	56.9	64.9	4.13	0.02
Leu	1.53	1.44	0.10	0.51
Lys	209	185	16.7	0.24
Met	17.6	17.3	1.20	0.88
Orn	111	106	9.34	0.54
Phe	52.7	50.1	2.58	0.47
Pro	62.4	67.4	3.20	0.05
Ser	57.4	55.0	3.53	0.48
Thr	148	133	8.83	0.11
Trp	18.9	27.7	1.91	<0.01
Tyr	42.4	47.7	2.99	0.10
Val	254	248	15.3	0.61

[1]Continuous abomasal infusions of L-Trp (0 vs 1 g/d) dissolved in an essential AA solution.
[2]Standard error of the mean (n = 15).

Table 4. Effects of energy infusion on nutrient intake, digestibility and N retention of growing lambs

	Energy, Mcal GE[1]			*P*-
Item	0	0.4	SEM[2]	value
Intake, g/d				
OM	734	734	0.20	0.40
NDF	458	458	0.09	0.40
N[3]	28.6	28.6	0.005	0.40
Fecal, g/d				
OM	117	114	5.69	0.66
NDF	70.3	68.3	3.51	0.56
N	5.35	5.15	0.26	0.46
Urinary, g/d				
N	12.9	10.6	0.64	<0.01
Digestibility, %				
OM	84.1	84.4	0.77	0.66
NDF	84.6	85.1	0.77	0.56
N	81.3	82.0	0.89	0.46
Retention				
N, g/d	10.3	12.6	0.77	<0.01
N, %	36.0	44.2	2.70	<0.01

[1]Continuous ruminal infusions of acetate (35 g/d) and propionate (10 g/d), plus abomasal glucose (60 g/d) infusions (0.4 Mcal GE/d) vs water infusion (0 Mcal GE/d).
[2]Standard error of the mean (n = 15).
[3]Dietary intake + abomasally infused.

Table 5. Effects of energy infusion on serum metabolites and plasma AA in growing lambs

	Energy, Mcal GE[1]			*P*-
Item	0	0.4	SEM[2]	value
Serum				
Urea N, mg/dL	12.1	11.0	0.60	0.02
Glucose, mg/dL	72.6	76.3	2.13	0.23
Insulin, ng/mL	0.69	0.83	0.10	0.15
IGF-I, ng/mL	116	110	9.6	0.35
Plasma AA, μ*M*				
Ala	133	114	5.68	0.01
Asn	29.3	27.5	1.59	0.22
Asp	4.05	3.74	0.23	0.34
Cys	0.98	1.04	0.06	0.50
Gln	314	292	12.5	0.07
Glu	73.3	62.4	3.86	0.05
Gly	485	561	36.8	0.02
His	66.1	66.5	4.04	0.92
Ile	66.1	55.8	4.13	<0.01
Leu	1.56	1.42	0.10	0.27
Lys	207	187	16.7	0.32
Met	17.9	17.0	1.20	0.58
Orn	113	104	9.34	0.30
Phe	50.7	52.1	2.58	0.70
Pro	68.0	61.8	3.19	0.02
Ser	51.9	60.5	3.53	0.02
Thr	138	143	8.83	0.63
Trp	24.1	22.5	1.91	0.40
Tyr	45.7	44.4	2.99	0.68
Val	261	240	15.3	0.07

[1]Continuous ruminal infusions of acetate (35 g/d) and propionate (10 g/d), plus abomasal glucose (60 g/d) infusions (0.4 Mcal GE/d) vs water infusion (0 Mcal GE/d).
[2]Standard error of the mean (n = 15).

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CONCEPTION RATE OF BEEF FIRST CALF HEIFERS SUPPLEMENTED WITH DIFFERENT PROTEIN CONCENTRATES IN THE TROPICS

R. E. Mora[1], C. F. Chicco[2], S. Godoy[3], A. M. Herrera[1], A. E. Darghan[1]
Universidad Nacional Experimental del Táchira (UNET)[1], Universidad Central de Venezuela (UCV)[2], Instituto Nacional de Investigaciones Agrícolas (INIA-CENIAP)[3]. Venezuela.

ABSTRACT: To evaluate different protein supplements, an experiment was carried out in the western plains of Venezuela with Brahman crossbred first calf heifers. The animals (n=120) of 3 and 4 years of age, having on average 385 ± 29.4 kg BW prior calving were divided in three uniform groups by BW and time of calving and assigned to three supplements (1 kg/d), each containing mainly corn meal (CM), hydrolyzed feather meal (HFM) and soy bean meal (SBM). The supplement had 21, 42.7 and 42.5% CP, and 3.10, 2.88, and 2.95 Mcal ME/kg, respectively for CM, HFM and SBM. Animal grazed in three *B. humidicola* pastures, in a rotational system, every 28 days. Stocking rate was 0.44 animal/ha. Animals had free access to water and mineral mix. Forage samples were taken every 28 days for chemical analyses. Supplementation started 45 days prior calving and was maintained up to the end of the breeding season. First calf heifers were bred by bulls in a 20:1 ratio. Pregnancy was determined by transrectal palpation. Pre-partum and pre-breeding periods lasted 44 ± 20 and 79 ± 25 days, respectively, with a breeding season of 108 days. Data were analyzed by least squares, including in the model BW, age, and supplementation time. Grass had 2.6 ± 0.5% CP and 77.5 ± 2.2% NDF. Daily avg DM available forage was 55 kg/animal. At calving, animals lost weight in all treatments, with no difference between them. From calving and beginning of the breeding season, SBM animals gained (g/d) more weight (301; $P<0.05$) than HFM (46) while CM lost weight (-27). Calving rate was 60, 63.6, and 50% respectively, with no difference between them. No differences were found for calf weights at birth in all treatments. Similar trend was found when 3 and 4 years old cows were independently analyzed. The latter had heavier calves ($P<0.05$) than the former (32.9 vs 30.9 kg). It is concluded that protein sources had no effect on conception rate. However, protein supplements reduced body losses when compared with corn meal supplement.

Key words: first calf heifers, protein sources, conception rate

Introduction

Cattle production in Venezuela is based mainly upon grazing systems and the quality and quantity of available forage depend on soil fertility and rain fall seasonal variations. Therefore, animals are subject to seasonal nutrient deficiencies that have a negative effect on production and reproduction performance. Supplementation is not a common practice. This is especially important for strategic animals of the herd, such as heifers and, particularly, for first calf heifers which are still under nutritional stress for growth and lactation (Ciccioli et al., 2003). As a consequence, conception rate is particularly low at breeding time (Sasser et al., 1988; Short and Adams, 1988; Randel, 1990). Under tropical conditions, protein is one of the most important limiting nutrients for cattle performance. Limited ammonia nitrogen in the rumen system impairs adequate microbial activity, with a negative effect on digestibility and transit of ingested materials and, as a consequence, limiting forage intake (Preston and Leng, 1987). Therefore, the objective of this research was to evaluate the effect of supplements with two protein sources of different ruminal degradation rate upon body weight changes and pregnancy of Brahman cross bred first calf heifers grazing tropical forages.

Materials and Methods

The experiment was carried out in the south - western plains of Venezuela, at 100 m.a.s.l., characterized by heavy-clay and poorly drained soils, partially floated during the rainy season, corresponding to the dry tropical forest ecological system (Holdridge, 1967). One hundred and twenty Brahman cross bred first calf heifers, averaging 385 ± 29.4 kg BW of 3 (n=93) and 4 (n=27) years old, were uniformly assigned by BW, reproductive condition, age, and time of calving to three supplemental treatments, containing each mainly corn meal (CM), hydrolyzed feather meal (HFM) and soy bean meal (SBM). The supplement (1kg/animal) contained 21, 42.7 and 42.5% CP, and 3.10, 2.88, and 2.95 Mcal ME/kg, respectively, for CM, HFM and SBM (Table 1).

Table 1. Experimental supplements composition for grazing first calf heifers

Ingredients	CM (%)	HFM (%)	SBM (%)
Urea	2.5	2.5	2.5
Ammonium sulfate	4	4	4
Minerals[1]	5	5	5
Undegradable fat	8	8	8
Molasses	5	5	5
Corn meal	75.5	45	15
Hydrolyzed feather meal	-	30.5	-
Soy bean meal	-	-	60.5
Nutrients			
Crude Protein [2]	20.1	42.7	42.5
Degradable Protein[2]	14.8	21.1	31.5
Undegradable Protein[2]	5.3	21.6	11.0
ME, Mcal/kg[2]	3.16	2.88	2.95

[1]Content (%): Ca, 21.6; P, 11.03; Mg, 2.02; Na, 2.65; Cu, 0.04; Zn, 0.17; Fe, 0.15; Mn, 0.17.
[2] Calculated values.

Animal grazed in three *Brachiaria humidicola* pastures, in a rotational system, every 28 days to minimize pasture effects. Stocking rate was 0.44 animal/ha. Animals had free access to water and mineral mix. Forage samples were taken every 28 days for chemical analyses. Supplementation started 45 days prior calving and was maintained up to the end of the breeding season of 108 days long. The experiment initiated at the end of the rainy season, while the breeding period lasted up to the transition of the dry-wet season.

Body weight was measured at the beginning of the experiment, at calving and at the beginning and end of the breeding period. First calf heifers were bred by bulls in a 20:1 ratio. Semen quality was evaluated to ensure sperm quality. Pregnancy was determined by transrectal palpation during the breeding season and 54 days after.

Available forage DM was measured on monthly basis, with a 0.375 m² metallic frame ad random on linear transepts. Forage sample were analyzed for CP (AOAC, 1990), NDF and ADF (Van Soest and Wine, 1967) and P (Chen et al., 1956).

Data were treated by ANOVA and covariance analyses to evaluate the effect of supplements on BW changes, in a completely randomized design corresponding to a 2x3 unbalanced factorial arrangement (2 ages; 3 treatments). To evaluate treatment effect on pregnancy, an exact logistic regression model was used (Mehta and Patel, 1995), including age and supplementation time effects.

Results and Discussion

Pre-partum and pre-breeding periods lasted 44 ± 20 and 79 ± 25 days, respectively, with a breeding season of 108 days. Average stocking rate was 0.44 animal/ha and available forage DM was 3274, 5658 and 5025 kg/ha at the beginning of the experiment, and at the beginning and at the end of the breeding season, respectively.

On average, daily available forage dry matter was 55 kg/animal. Available dry matter was higher than the values of 2000 kg/ha suggested by Minson (1990) or the 30 kg/animal/day as indicated by Lamela (1992). However, forage CP content decreased (P<0.05) progressively along the three periods, and was below the value of 7% suggested by Milford and Minson (1966), as the lower limit to ensure adequate forage intake, including the added CP provided by the supplements.

Table 2. Seasonal variation of nutrient content of forage samples

Periods	CP	NDF	ADF	P
	%			
Transition rainy-dry season (Beginning of experiment)	3.04 ± 0.57^{a}	75.59 ± 1.86^{b}	44.93 ± 3.04^{b}	0.16 ± 0.02^{a}
Dry season (Beginning of breeding season)	2.57 ± 0.29^{ab}	78.45 ± 1.25^{a}	48.00 ± 0.64^{a}	0.16 ± 0.02^{a}
Transition dry-rainy season (End of breeding season)	2.29 ± 0.39^{b}	78.65 ± 2.8^{a}	48.12 ± 1.79^{a}	0.11 ± 0.04^{b}

[a, b] Numbers in the same column with different superscripts differ (P<0.05).

Differences among periods (P<0.05) were registered for NDF, ADF and P content. Forage structural elements increased along the experiment, while P content diminished, with values lower than 0.25% P, level considered as limiting for cattle production (McDowell, 1985). In addition, 5.5 g/day of P was provided by the supplements that in no case was sufficient to supply minimum requirements.

Supplements had no effect on calf weights at birth and cows weights at calving time. On the other hand, supplements had a significant (P<0.05) effect on BW of first calf heifers between calving and beginning of the breeding season. While HFM and SBM animals gained weight (46.87 and 301.29 g/d), CM animals lost weight (-27.32 g/d). The greater (P<0.05) effect of SBM is probably due to a greater release of ammonia nitrogen in the rumen system (Anderson et al., 2001) when animals graze very poor forage. However, between the beginning and the end of the breeding period, all animals lost weight, with no difference between treatments. This is probably due to a decrease of forage CP that was not compensated by supplements.

Conception rate was no affected (P>0.05) by the different protein supplements (63.6 and 60%) and also when these were compared with corn supplemented animals (50.0%). Similar results were reported in first calf Brahman heifers by Vásquez and Bastidas (2005) with two protein supplements that contained fish meal and cotton seed meal, with 56 and 50% pregnancy, respectively, that were significantly higher than the unsupplemented control group, with 25% pregnancy. Alderton et al. (2000) and Anderson et al. (2001) also reported no effect of two types of protein supplements (degradable and undegradable protein in the rumen) on pregnancy of first calf beef heifers.

When, independently of treatments, 3 and 4 years old animals were compared, adjusted data for number of animals and time of supplementation, showed that 4 years old first calf heifers had heavier calves (P<0.05) at birth (32.90 kg) than 3 years old animals (30.97 kg). This is due to a greater weight of the older animals (409.8 vs 376.9 kg), which is a common finding in the literature (Knapp et al., 1940; Braude. and Walker, 1949; Pálsson, 1959).

Table 3. Supplementation time, adjusted body weight, and pregnancy rate of grazing first calf heifers supplemented with different degradable protein sources

Items	Supplements		
	CM	HFM	SBM
Initial body weight, kg	385.07 ± 30.5[a]	386.88 ± 30.3[a]	385.83 ± 28.9[a]
Pre-calving supplementation, d	49 ± 21[a]	43 ± 22[a]	39 ± 17[a]
Supplementation calving-breeding, d	32 ± 25[a]	39±31[a]	40 ± 28[a]
Total supplementation time, d	189 ± 21[a]	190±22[a]	188 ± 24[a]
Calf birth weight, kg	31.68[a]	32.20[a]	32.16[a]
Cow BW at calving, kg	340.51[a]	337.06[a]	342.06[a]
Cow BW changes from calving to breeding, g/d	-27.31[b]	46.87[ab]	301.29[a]
Cow BW changes from beginning to end breeding season, g/d	-200.87[a]	-193.47[a]	-153.10[a]
Body weight at end of the breeding season, kg	324.03[b]	323.38[b]	338.63[a]
Pregnancy, %	50.0[a]	63.6[a]	60.0[a]

[a, b] Numbers in the same row with different superscripts differ (P<0.05).

After calving, 3 and 4 years old animals gained weight with no difference due to age. However, since the beginning of the breeding season 3 and 4 years old first calf heifers lost weight, being this grater in younger animals (-229.67 vs -135.29 g/day) This finding is probably due to that older animals have lower nutrient requirements for growth (Ducker et al., 1985).

Four years old animals had grater (P<0.05) pregnancy rate (70.4%) than younger animals (53.6%). Short et al. (1990) indicate that younger animals have a lower reproduction potential due to a prolonged postpartum anestrous.

Table 4. Supplementation time, adjusted body weight, and pregnancy rate of 3 and 4 years old first calf heifers supplemented with different degradable protein sources

Items	Age (years)	
	3	4
Initial body weight, kg	376.97 ± 26.3[b]	409.88 ± 24.2[a]
Pre-calving supplementation, d	47 ± 20[a]	33 ± 17[b]
Supplementation calving-breeding, d	28 ± 24[b]	60 ± 27[a]
Total Supplementation time, d	184 ± 22[b]	202 ± 20[a]
Calf birth weight, kg	30.90[b]	32.97[a]
Cow BW at calving, kg	327.77[b]	351.99[a]
Cows BW changes from calving to breeding, g/d	138.80[a]	75.11[a]
Cows BW changes from beginning to end breeding season, g/d	-229.67[b]	-135.29[a]
Cow BW at end of the breeding season , kg	319.17[b]	338.18[a]
Pregnancy, %	53.6[b]	70.4[a]

[a, b] Numbers in the same row with different superscripts differ (P<0.05).

Implications

It is concluded that first calf cross bred Brahman grazing poor quality forages, under tropical conditions, limited supplements with different protein sources had no effect on conception rate. However, protein supplements reduced body losses when compared with corn meal supplement. Older animals showed better body weight and pregnancy performance

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THE FEED VALUE OF DRY DISTILLERS GRAINS PLUS SOLUBLES IN 90% CONCENTRATE DIETS FOR FEEDLOT LAMBS

G. Orosco, M. F. Martinez-Perez, Y. Diaz, J. M. García-Madrid, A. Islas, and S. A. Soto-Navarro*.

Department of Animal and Range Science, New Mexico State University, Las Cruces, NM, USA

ABSTRACT: Twenty four Rambouillet lambs (31.3 ± 0.77 kg initially) were used in a 92-d finishing experiment in order to evaluate the feed value of dry distillers grains plus solubles (DDGS) in 90% concentrate diets. Four concentrations (8, 16, 24, and 32% of diet DM) of DDGS replaced dry-rolled corn in a 90% corn-based finishing diet. Lambs were housed individually and fed once daily at 0800. Fresh water was always available. Average daily gain tended to increase with increasing DDGS level (P = 0.09, quadratic; 261, 288, 280 and 257 ± 13.9 g/d for 8, 16, 24, and 32% DDGS, respectively). Dry matter intake (1,304, 1,256, 1,326 and 1,318 + 74.2 g/d for 8, 16, 24 and 32% DDGS, respectively), and G:F (181, 193, 198, and 188 ± 9.9 g/kg for 8, 16, 24, and 32% DDGS, respectively) were not affected ($P \geq 0.39$) by DDGS replacement level. Hot carcass weight, LM area, backfat thickness, and marbling score were not affected ($P \geq 0.18$) by increasing DDGS supplementation level. Although quality grade decreased (P = 0.03, linear) with increasing DDGS supplementation level, the average carcass quality grade in each treatment was within the choice category. We conclude that growth performance of lambs consuming 90% concentrate diets based on corn is not affected by level of DDGS replacement. Therefore, DDGS can be used in 90% concentrate diets based on corn up to 32% without negatively affecting the feed value of the diet.

Keywords: DDGS, feedlot lambs, performance

Introduction

The ethanol industry is expanding rapidly and by-products such as corn dried distillers grains with solubles (**DDGS**) are becoming widely available (Renewable Fuels Association, 2005). Corn dried distillers grains with solubles are relatively high in CP (20%), fat (12%), NDF (36%), and P (0.9% of DM). Also, DDGS are competitively priced compared with other protein and energy sources. The use of DDGS as a feedstuff for livestock has been documented from as early 1900 (Henry, 1900). Successful use of DDGS have been reported for feedlot cattle (Ham et al., 1994), grazing cattle (MacDonalds et al., 2007), creep-fed calves (Reed et al., 2006), and dairy cattle (Al-Suwaiegh et al., 2002). In a meta-analysis of 4 studies (Klopfenstein et al., 2007) reported that the feeding value of DDGS was 123% of that of corn when the level of inclusion in a finishing diet was 20%, and declined to 100% with increasing the level of inclusion to 40%. In a previous study conducted by our group it was found that the feeding value of DDGS was similar to corn when fed to lambs consuming 80% concentrate corn-based diets (Diaz et al., 2008). Since ruminal pH decreases with increasing cereal grains in diets (Britton and Stock, 1987), we hypothesize that DDGS (high-fiber ingredient) digestibility will decrease at higher cereal grain concentrations of the diets (Russell and Wilson, 1996). Lower digestibility of DDGS will result on poorer growth performance. However, information on uses of DDGS as feedstuff for lambs consuming 90% concentrate diets is limited. Therefore the objective of this study was to evaluate the effect of level of DDGS inclusion on 90% concentrate diets based on dry rolled-corn on growth performance.

Materials and Methods

Twenty four spring-born Rambouillet wether lambs born on the main campus at New Mexico State University were used in this study. All procedures were approved by the Institutional Animal Care and Use Committee. Lambs were docked at 1 d of age, castrated and vaccinated against tetanus and enterotoxemia at 28 d of age and again at weaning when lambs were approximately 60 d of age. During the preweaning period, lambs had free access to alfalfa hay and cracked corn was offered at levels appropriate for age and BW. After weaning, lambs were fed alfalfa hay and cracked corn until they were approximately 80 d of age at which time they began an adaptation period to the basal experimental diet. When lambs weighed 31.3 ± 0.77 kg and were 103 ± 1.2 d of age, they were stratified by BW and randomly assigned to 1 of 4 dietary treatments. During the 92-d experiment, lambs were maintained outdoors in individual pens (2 x 4 m) and had free access to water and their experimental diet.

All diets were composed of 9.8% sudangrass as the roughage source. The basal diet contained 72.2% dry-rolled corn, and 8% DDGS yielding a CP content of 14.6%. Dicalcium phosphate and limestone were added to balance the P and Ca contents among dietary treatments. The remaining diets had 16, 24, and 32% DDGS replacing dry-rolled corn, to yield respective CP (DM basis) levels of 15.3, 16.0, and 16.7%. Other dietary ingredients were incorporated in similar amounts in all diets and included molasses (3.0%), tallow (approximately 0.5%), ammonium chloride (1.0%), salt (1.0%), and a vitamin premix (0.5%; 2,200 IU/g vitamin A, 1,200 IU/g Vitamin D_3, and 2.2 IU/g vitamin E). The ratio of degradable intake protein to TDN was 0.115 for each of the diets, urea was used to achieve such ratio. Fresh feed was offered daily in amounts to

stimulate ad libitum intake and orts were recorded daily. Orts were pooled weekly and DM was determined. Lambs were weighed at 21-d intervals.

The HCW were obtained from all lambs at time of harvest. After carcasses were chilled for 48 h the following measurements were obtained: 1) LM area, taken by direct grid reading of the muscle at 12th rib; 2) subcutaneous fat over the LM at the 12th rib; 3) KPH as a percentage of HCW; and 4) marbling score (USDA, 1992).

Data were subjected to ANOVA appropriate for a randomized complete block design with animal as the experimental unit. Analyses were computed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment was used as fixed effect and block as random effect. Because treatments were arranged with increasing levels of DDGS, orthogonal contrasts were used to test for linear, quadratic, and cubic responses.

Results and Discussion

Average daily gain tended to increase with increasing DDGS level (P = 0.09, quadratic; 261, 288, 280 and 257 ± 13.9 g/d for 8, 16, 24, and 32% DDGS, respectively). Dry matter intake (1,304, 1,256, 1,326 and 1,318 + 74.2 g/d for 8, 16, 24 and 32% DDGS, respectively), and G:F (181, 193, 198, and 188 ± 9.9 g/kg for 8, 16, 24, and 32% DDGS, respectively) were not affected ($P \geq 0.39$) by DDGS replacement level. Hot carcass weight, LM area, backfat thickness, and marbling score were not affected ($P \geq 0.18$) by increasing DDGS supplementation level. Although quality grade decreased (P = 0.03, linear) with increasing DDGS supplementation level, the average carcass quality grade in each treatment was within the choice category.

The feeding value of DDGS for beef feedlot diets have been previously reported and date of 4 studies were summarized by Klopfenstein et al. (2007). That meta-analysis reported that the feeding value of DDGS was 123% of that of corn when the level of inclusion in a finishing diet was 20%, and declined to 100% with increasing the level of inclusion to 40%. However, in agreement with the present study, the feeding value of DDGS in lamb finishing diets has been found to be similar to that of dry-rolled corn Lodge et al., 1997; Diaz et al., 2008). It was hypothesized that DDGS would have lower feeding value in relation to that of corn when included in diets with greater cereal grain concentration. The rezoning was that greater cereal grain concentration results on lower ruminal pH (Britton and Stock, 1987), which has a negative impact on fiber digestion (Russell and Wilson, 1996). A considerable proportion of energy from DDGS is derived from it high fiber content (36%; Stock et al., 2000). However, we fail to test that hypothesis, since the feeding value for DDGS in the present study (90% concentrate diet) and with 80% concentrate diets (Diaz et al., 2008) was similar to that of dry-rolled corn. More research if required to investigate the feeding value of DDGS when included at less concentrate diets.

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Table 1. Influence of DDGS level on performance and carcass characteristics of Rambouillet lambs consuming a 90% concentrate diet

	DDGS level, %[a]						Contrasts[b]		
Item	8	16	24	32	SE[c]	*P*-value	Linear	Quadratic	Cubic
Initial BW, kg	31.4	31.0	31.5	31.2	0.77	0.88	0.97	0.93	0.43
Final BW, kg	55.4	57.5	57.2	54.9	1.35	0.45	0.80	0.12	0.97
ADG, g/d	281	288	280	257	13.9	0.37	0.18	0.09	0.74
DMI, g/d	1304	1256	1326	1318	74.2	0.39	0.61	0.70	0.23
G:F ratio	181	193	198	188	9.9	0.41	0.54	0.15	0.63
HCW, kg	28.1	28.7	29.8	27.2	1.29	0.56	0.81	0.24	0.48
LM area, cm^2	14.5	13.7	13.9	13.7	0.46	0.57	0.29	0.50	0.54
Fat thickness, cm	0.57	0.64	0.60	0.51	0.07	0.46	0.40	0.19	0.90
Body wall, cm	1.6	1.97	1.84	1.91	0.27	0.31	0.25	0.30	0.25
Quality grade[d]	11.5	11.5	11.0	10.5	0.32	0.14	0.03	0.45	0.74
Marbling score[e]	412.5	425	375	407.5	29.7	0.57	0.58	0.70	0.23

[a]DDGS treatments consisted of 4 concentrations of DDGS (8, 16, 24 and 32% DM basis) replaced dry-rolled corn in a 90% corn-based finishing diet.
[b]Standard error of treatment means; n = 6 lambs per treatment.
[c]Probabilities for the linear, quadratic, and cubic effects of level of DDGS.
[d]Coded: low choice = 10, average choice = 11, and high choice = 12.
[e]300 = Slight 00; 400 = Small 00; 500 = Modest 00.

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Effects of Time of Transporting Prior to Sale Date on Selling Weight of Weaned Steer Calves

L. M. Kovarik*, M. K. Luebbe, J.R. Benton, G. E. Erickson, and R. J. Rasby
University of Nebraska-Lincoln

ABSTRACT: Eighty-eight crossbred weaned steer calves (250 ± 26 kg) were used to evaluate weight loss (shrink) when transported to a mock sale barn on differing days prior to sale. The calves were weaned 14 days and fed free choice grass hay and 0.9 kg of DDGS at UNL's Dalbey-Halleck Research Unit. Two days before initiation of treatments all calves were fed grass hay ad libitum and initial BW were recorded on two consecutive days. Calves were assigned randomly to treatment as a completely randomized design. On d 2, 1d restricted and 1d ad libitum treatments were transported 150 km to UNL ARDC feedlot near Mead, Nebraska. 0d treatment remained at the Dalbey-Halleck Research Unit. All treatments were allowed free choice grass hay and water. At 0800 on d 3, 0d treatment was loaded into trailers and transported to the ARDC feedlot. At 0800 on d 3, 1d restricted treatment was withheld from grass hay and water. At 1000 on d 3, all calves were co-mingled and processed to obtain a sale BW. The mixed procedures of SAS were used for statistical analysis, with steer as the experimental unit. No differences were observed in final BW (P=0.98) or percent shrink (P=0.80) with 2.2%, 1.7%, and 0.6% shrink for 1d restricted, 1d ad libitum and 0d treatments, respectively. Total weight loss from two days pre-mock sale date to the mock sale date was 7.0, 6.0, and 1.8 kg for 1d restricted, 1d ad libitum and 0d treatments, respectively. Transporting date prior to sale date did not significantly affect selling weight when calves were allowed access to feed and water at the sale facility and had been trained to eat hay prior to the sale date. However, a numerical difference was observed between shipping cattle prior to sale date.

Key Words: Shipping, Shrink, Weaning

Introduction

Sale weight has a large impact on the price received for the livestock traded. Many factors such as diet, age, weaning status, and pen conditions can affect sale weight. In a study conducted by Self and Gay (1972) cattle were shipped from 53 different locations and the distances varied from 240 to 1,824 km with the average being 1,024 km. Cattle were either shipped directly from the ranch or from a sale barn. The average amount of shrink was 7.2% and 9.1% respectively and significantly different (P= 0.05). The objective of this study was to evaluate the effects of time of transporting prior to sale date on selling weight of weaned steer calves.

Materials and Methods

Eighty-eight crossbred steers were randomly assigned to one of three treatments. Steer calves were weaned for 14 d at UNL's Dalbey-Halleck Research Unit near Virginia, Nebraska. Calves received 0.9 kg of DDGS and free choice grass hay during the weaning phase. Initial weights were taken on d 1. Day 2 second day weights were recorded and calves were randomly assigned to treatment. 1d restricted (n=29), and 1d ad libitum (n=29) were transported 150 km to ARDC research feedlot near Mead, Nebraska. 0 d calves (n=30) remained at the Dalbey-Halleck Research Unit. All three treatments received free choice grass hay. On d 3 at 0800 d 1 restricted were removed from hay and water. 1 d ad libitum was allowed access to feed and water. Also, 0 d were transported to ARDC research feedlot in stock trailers. Upon arrival calves were co-mingled and processed. The weights recorded at processing were used as sale weights. Data were analyzed using the mixed procedures of SAS.

Results

Initial BW did not differ (P = 0.07) and was 256, 251, and 241 kg for 1d restricted, 1d ad libitum, and 0d treatments, respectively. No differences were observed in final BW (P=0.33) with 249, 245, and 239 kg or percent shrink (P=0.80) with 2.2%, 1.8%, and 0.6% shrink for 1d restricted, 1d ad libitum and 0d treatments, respectively. Total weight loss from two days pre-mock sale date to the mock sale date was 7.0, 6.0, and 1.8 kg for 1d restricted, 1d ad libitum and 0d treatments, respectively. No statistical differences were observed when comparing treatments.

Discussion

Shrink is a highly variable physiological process with the contents of the digestive system being highly affected. Sixty steers were slaughtered pre and post-shipment to provide information on the source of shrink (Self and Gay 1972). Slightly less than half of total shrink was from loss of digestive tract contents. The mean time required to regain shipping shrink was 10.7 days. However, the time required to regain shrink ranged from 3 to 30 days with over half the shipments only requiring 7 days or less. Also, environmental stressors such as high ambient temperatures and excessive handling increases shrink by up to 2% (Coffey et al. 2001).

In a study conducted by Coffey et al. (1997) they found that yearling steers gathered at 6 a.m. vs. 9 a.m. had 2.9% greater shrink at 3 p.m. Researchers attributed this

finding to the extra time that cattle remained on the pasture and their opportunity to graze and consume water. In addition to time of gathering, diet can play an important role on shrink. Cravey et al. (1991) compared shrink when cattle where grazing wheat pasture or were eating hay in a dry-lot. After four hours, wheat pasture cattle had shrunk 5.1% vs 3.9% for cattle consuming hay in the dry-lot.

Also, in recently weaned and transported calves, low feed intake is common and may persist for up to 2 weeks (Hutchinson and Cole, 1986).

According to Barnes et al. (1990) shrink was greatest in calves weaned the day before the sale when compared to calves weaned the day of the sale or 22 days preconditioned: 4.9, 3.4, and 2.3% respectively. In review conducted by Coffey et al. (2001) it was stated that the primary factor affecting shrink is the length of time feed and water are restricted. The average shrink is 1%/hr during the initial 3-4 hours but then decreases to as low as 0.1%/hr after 10 hr or more.

In our trial the objective was to discover the amount of shrink recovered or lost in twenty-four hours at a new location with weaned calves that are preconditioned to eating hay. We hypothesized that calves shipped on 1d would gain back the shrink incurred in the shipping process. However, in our data, the 1d calves continued to shrink in the new environment. The 1 d restricted calves shrunk more than 1 d ad libitum calves. The 0 d calves lost numerically the least amount of weight.

Implications

Our data were not statistically different between treatments but the numerical differences in our shipping procedures could have an economic importance. Shipping date prior to the sale does not statistically affect sale weight. Other economic variables need to be accounted for to decide the date to ship cattle prior to sale date.

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Table 1. Effects shipping time prior to sale.

	Treatment[1]				
Performance Characteristics	1 d restricted	1 d ad libitum	0 d	SEM	*P*-VALUE
Initial BW, kg	256.7	251.7	241.3	10.5	0.07
Final BW, kg	249.7	245.7	239.5	10. 7	0.33
% shrunk, (1- (FBW / ItBW))	2.2	1.8	0.6	0.02	0.80

[1]Ttreatments: 1 d restricted = transported 1 day prior to sale and restricted for 2 hours; 1 day ad libitum = transported 1 day prior to sale and allowed ad libitum access to feed and water; 0 day = Transported the day of the sale.

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PERFORMANCE OF EWE LAMBS FED OAT HAY

D. Domínguez [1], S. Ramírez[1], J. J. Salmerón [2], R. Ochoa[2], G. Villalobos[1], H. Castillo[1] and J. Ortega[1]
[1]Universidad Autónoma de Chihuahua, Chihuahua, México.
[2]INIFAP, Cuauhtémoc, Chihuahua, México.

ABSTRACT: Oat hay is one of the main forage sources for the cattle industry in Chihuahua, México. However, there is scarce information regarding nutritional value of this forage. This study evaluated the effect of genotype and maturity stage on nutritional value of oat hay and its impact on performance of ewe lambs. For this purpose Cuauhtémoc, Menonita and Bachíniva oat varieties were cultivated under non irrigated conditions and harvested at soft-dough (SDS) and hard grain stage (HGS). Seventy two ewe lambs with an average weight of 19.5 +/- 3.8 kg were fed ad libitum during 63 d a diet containing 63.4 to 61.3, and 36.6 to 38.7 % of oat hay and concentrate on dry matter basis for SDS and HGS, respectively. Production of dry matter per hectare (DM/ha), and content of CP, NDF, ADF, LDA and dry matter digestibility (DMD) were determined for oat varieties. Dry matter intake (DMI) was determined daily, body weight, average daily gain (ADG), gain efficiency (GE) were determined every 21 d, and apparent dry matter digestibility at the final of the experiment. Data was analyzed using the PROC MIXED of SAS. There was no effect of oat variety and maturity stage on DM/ha and chemical composition of oat hay. Dry matter production was 5740 and 5959 kg/ha for SDS and HGS, respectively. Content of CP, NDF, ADF, LDA and DMD for SDS and HGS were: 9.4 and 8.4, 55.4 and 52.6, 31.6 and 29.5, 3.2 and 3.0, and 64.3 and 65.9%, respectively. Similarly, DMI, final body weight, ADG and GE were similar among treatments, averages for SDS and HGS were: 1.19 and 1.16, 35.3 and 35.5, 0.109 and 0.120, 11.4 and 10.7 kg, respectively. Apparent dry matter digestibility was not affected by treatments, average for SDS and HGS were 62.7 and 61.5%, respectively. Harvesting and feeding oat hay at SDS did not improved nutritive value of forage, as well as animal performance.

Key Words: oat hay, maturity stage, ewe lambs.

Introduction

Oat hay is the main forage source for livestock industry in the west of Chihuahua, where approximately 200,000 hectares are cultivated with oat under non irrigated conditions (SIAP, 2009). Typically oat is harvested at hard grain stage in order to maximize biomass production, achieving 5,000 kg/ha (Domínguez et al., 2008b), but nutritional quality is reduced due to a higher fiber concentration and lower content of CP (Collar et al., 2004). However, research trials have been showed that harvesting oat at hard grain stage allows for its highest grain content, leading to a deep fiber dilution effect and a better nutritional value.

Sheep production system in Chihuahua therefore, feeding animals with oat hay varying in its harvest stage could affect their performance. Kraiem (1997) reported 17.1% higher DMI in rams fed a 60:40 forage:concentrate diet, when the oat hay used was cut at soft dough stage compared to hard grain stage.

Oat is commonly used in feeding programs in the sheep production system in Chihuahua. However, there is no information respecting the genotype and maturity stage of oat on animal performance.

The objective was to evaluate the effect of oat genotype and maturity stage on performance of lamb ewes.

Materials and Methods

This study was conducted in the facilities of INIFAP at Research Center of Sierra de Chihuahua in Bachíniva, Chihuahua, México. In June of 2006, Cuauhtémoc (CUA), Menonita (MEN) and Bachíniva (BAC) oat varieties were cultivated under non irrigated conditions and harvested at soft dough (SDS) and hard grain stage (HGS) at 75 ± 8 and 88 ± 6 d after sowing, respectively. At harvest time oat forage samples were obtained to estimate dry matter production, then they were dried at 60 °C during 48 h to determine DM content, then they were ground at 1mm to determine absolute DM, OM, CP (AOAC, 1995) and NDF, ADF (Goering and Van Soest, 1970) and LDA (Van Soest et al., 1991) sequentially using the ANKOM^{200} fiber analyzer. Dry matter digestibility of oat forages were estimated using the equation of Moore and Undersander (2002). Oat forage harvested was baled and then ground at 2.5 cm of theoretical length of cut.

Seventy crossbred two ewe lambs of commercial with an average weight of 19.5 +/- 3.8 kg were treated for internal and external parasites and vitamins A; D and E were applied prior to the beginning of the experiment, allowing for 10 d of adaptation period. Animals were randomly assigned into 24 different groups of 3 individuals of similar initial body weight, corresponding to the 6 experimental treatments (n=4). Lambs were fed ad libitum isoenergetic and isonitrogenous rations once daily at 0730 h

(Table 1) during 63 d containing 63.1 to 61.0, and 36.8 to 38.7 % of oat hay and concentrate on dry matter basis for SDS and HGS, respectively. Lambs were weighted every 21 d, and DMI was determined daily by pen. At the final of the experiment, apparent digestibility of DM was determined using indigestible ADF (IADF) as an internal marker (Penning and Johnson, 1983). For this purpose fecal samples were taken four times daily during three days from 16 lambs (n=4). The marker was determined in fecal, concentrate and oat forage samples placed in Ankom® bags F57(0.35 ± 0.5 g) and incubated in the ventral ruminal sac of two ruminal fistulated sheep during 12 d (Huhtanen et al., 1994). After this period bags were washed and ADF analysis was conducted.

Data collected from animal performance were analyzed with PROC MIXED of SAS using a complete random blocking design with a factorial 3 x 2 arrangement, considering as fixed effects animal, oat variety and maturity stage, and random effects block and period. Chemical composition and digestibility of oat hay were analyzed with the PROC GLM of SAS using a 3 x 2 factorial design.

Table 1. Composition of experimental diets.

	Soft-dough stage			Hard grain stage		
Item	CUA	MEN	BAC	CUA	MEN	BAC
	---------- % of DM ----------					
Oat hay	63.1	63.1	63.1	61.0	61.0	61.0
Cottonseed meal	15.0	15.0	15.0	15.0	15.0	15.0
Soybean meal	8.7	8.7	8.7	12.1	12.1	12.1
Corn flakes	6.7	6.7	6.7	6.0	6.0	6.0
Corn gluten meal	2.0	2.0	2.0	2.0	2.0	2.0
Molasses	1.0	1.0	1.0	1.0	1.0	1.0
Soybean oil	1.0	1.0	1.0	0.5	0.5	0.5
Calcium carbonate	1.4	1.4	1.4	1.5	1.5	1.5
Premix	0.5	0.5	0.5	0.4	0.4	0.4
Salt	0.5	0.5	0.5	0.5	0.5	0.5
	--------- Chemical composition (% DM) ---------					
CP	17.4	18.1	18.0	17.5	19.8	17.8
NDF	42.5	43.1	41.4	39.4	32.4	41.6
ME, Mcal/kg DM	2.29	2.29	2.29	2.27	2.27	2.27
Ca, %	0.30	0.30	0.30	0.30	0.30	0.30
P, %	0.40	0.40	0.40	0.40	0.40	0.40

Results and Discussion

Dry matter production and chemical composition of oat forage. Production of DM was slightly superior when oat was harvested at HGS (5,960 vs.5, 740 kg/ha; $P<0.05$). Chemical composition of oat was not affected ($P>0.05$) by genotype, maturity stage and their interaction (Table 2). However, CP, NDF, ADF and LDA content of oat were reduced at HGS vs. SDS. This response is in agreement with the results of Domínguez et al. (2008a and 2008b) and has been related to the higher grain content which leads to the fiber dilution effect. As ADF concentration of oat was reduced at HGS, DMD was improved. MEN oat variety showed the best nutritional value at HGS, while CUA oat variety had the lower nutritional value.

Animal performance. Similarly, there was no effect of oat genotype, maturity stage or they interaction on final body weight, DMI, ADG and GE of ewe lambs (Table 3). Overall means for SDS and HGS treatment were 35.3 kg, 1.19 kg DM, 0.10 kg/a, 11.4 and 35.5 kg, 1.16 kg DM, 0.12 kg/a and 10.7, respectively. Lack of response to fed oat hay harvested at SDS or HGS to ewe lambs on performance, could be related to the similar chemical composition of oat forages, even though they were harvested at different maturity stages.

Similar results were obtained by Kraiem et al. (1997) when fed rams with 100:00 and 60:40 forage: concentrate diets, using oat hay harvested at soft dough and hard grain stages. In this study chemical composition of oat forages was similar, and DMI differences were attributed to concentrate level due to its negative effect on fiber digestibility (1.400 and 1.344 vs. 1.839 and 1.570 kg/a/d, respectively)

Implications

Harvesting oat hay at soft dough stage did not improve nutritional value. Oat hay Menonita variety had the best nutritional value at hard grain stage. Feeding ewe lambs with oat hay harvested at soft dough stage did not improve dry matter intake, average daily gain and gain efficiency. Therefore, is recommended to harvest and fed oat hay at hard grain stage

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Table 2. Chemical composition of oat forages.

	Soft-dough stage			Hard grain stage				*P value*		
Item	CUA	MEN	BAC	CUA	MEN	BAC	SEM[a]	var	edo	var*edo
DM, %	92.3	92.4	91.9	92.1	92.0	91.9	0.58	0.832	0.673	0.946
CP, % DM	8.9	9.7	9.7	6.9[b]	9.9[a]	8.5[ab]	0.70	0.060	0.109	0.322
NDF, % DM	55.3	55.8	55.0	57.1[a]	48.0[b]	52.5[ab]	2.00	0.141	0.108	0.096
ADF, % DM	31.3	31.8	31.6	32.5[a]	26.3[b]	29.8[ab]	1.28	0.132	0.076	0.067
ADL, % DM	3.1	3.3	3.2	3.2	2.9	3.0	0.13	0.968	0.176	0.321
Hemicel., % DM	24.7	24.7	24.0	25.3[a]	22.3[b]	23.3[ab]	0.79	0.156	0.228	0.230
Celulosa, % DM	28.5	28.8	28.7	29.6[a]	23.7[b]	27.1[ab]	1.16	0.098	0.073	0.059
DMD, % DM	64.6	64.1	64.3	63.6[b]	68.4[a]	65.7[ab]	0.99	0.131	0.076	0.067
IVDMD, % DM[b]	69.4	66.0	65.4	59.3	63.3	63.7				

[a] Standard error of the mean for 12 ewe lambs per treatment. var = variety. edo = maturity stage.
[b] Absolute means.
Within a row, means without a common superscript differ ($P < 0.05$).

Table 3. Effect of genotype and maturity stage of oat hay on performance of ewe lambs.

	Soft-dough stage			Hard grain stage			
Item	CUA	MEN	BAC	CUA	MEN	BAC	SEM[1]
BW, kg							
0 d	28.2	28.9	28.3	28.0	27.4	29.4	0.93
21 d	30.4	31.0	30.5	29.7	29.4	32.2	1.05
42 d	32.8	34.1	33.6	32.5	32.6	35.7	1.05
63 d	34.5	35.8	35.6	34.7	34.3	37.7	1.05
DMD, %	64.50[b]	62.85[bc]	60.68[cd]	54.88[e]	67.90[a]	61.89[bcd]	0.885
DMI, kg							
21 d	1.14	1.08	1.14	1.12	1.02	1.14	0.049
42 d	1.24	1.18	1.22	1.20	1.11	1.25	0.049
63 d	1.26	1.17	1.25	1.22	1.13	1.25	0.049
0 a 63 d	1.22	1.14	1.20	1.18	1.09	1.21	0.045
ADG[2], kg							
21 d	0.137	0.117	0.113	0.117	0.128	0.156	0.016
42 d	0.101	0.140	0.133	0.124	0.141	0.153	0.016
63 d	0.071	0.067	0.087	0.094	0.069	0.086	0.016
0 a 63 d	0.105	0.110	0.113	0.113	0.115	0.134	0.013
GE[3], kg							
21 d	8.7	9.2	10.2	9.6	8.5	7.8	1.71
42 d	12.0	8.9	9.3	10.8	8.4	9.0	1.71
63 d	15.6	16.0	13.8	14.8	15.5	13.4	1.71
0 a 63 d	12.0	11.3	11.0	11.6	10.7	9.9	1.46

[1] Standard error of the mean for 12 ewe lambs per treatment.
[2] Average daily gain.
[3] Gain efficiency.
Within a row, means without a common superscript differ ($P < 0.05$).

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THE EFFECT OF FEEDING AN EXOGENUS FIBROLYTIC ENZIME ON PERFORMANCE OF FINISHING LAMBS

D. Domínguez, J. E. Cruz, G. Villalobos, H. Castillo, L. Duran and E. Santellano
Universidad Autónoma de Chihuahua, Chihuahua, México.

ABSTRAC: Use of fibrolytic enzymes can enhance fiber digestion improving ruminant performance. This study evaluated the addition of a fibrolytic enzyme on the performance of fattening lambs. Thirty two male weaned lambs of commercial crosses with an average weight of 20.6 +/- 2.3 kg, and 90 days old were randomly assigned to four levels of Fibrozyme®: 0.0 (T-0.0), 0.025 (T-0.025), 0.05 (T-0.05) and 0.1 gr/kg of body weight (T-0.1) which were adjusted every fourteen days. Lambs were fed ad libitum a diet containing 2.5 Mcal of ME/kg MS and 21.4% CP from the start of the experiment until they reached 30 kg of body weight, then a second diet containing 2.9 Mcal of ME/kg MS and 15.9 % CP was fed to finalize the animals. Dry matter intake (DMI) was determined daily and individually, body weight, average daily gain (ADG) and gain efficiency (GE) were determined every fourteen days. Data were analyzed using the PROC MIXED of SAS. Three lambs were eliminated from the trial, due to health problems, therefore T-0.0, T-0.25 and T-0.1 had a n= 7, while T-0.05 had a n=8. There was no improvement of DMI by feeding the fibrolytic enzyme (1.73, 1.69, 1.82 and 1.79 kg/d, respectively). Final body weight was similar among treatments (41.6, 39.6, 41.3 and 40.4 kg, respectively). Addition of the enzyme did not affect ADG (0.336, 0.302, 0.316 and 0.302 kg/d, respectively). Therefore, GE was not affected by feeding the enzyme (6.4, 6.4, 7.3 and 7.6, respectively). Performance of fattening lambs was not improved by adding the fibrolytic enzyme at the fed levels.
Key Words: fibrolytic enzyme, lamb, finishing diet.

Introduction

Sheep production in México and the state of Chihuahua has been growing during the last five years near to 30 and 25% respectively (Arteaga, 2008). The sheep system in Chihuahua is oriented to the fattening of the lambs and their marketing to central México. Commonly finishing diets for lambs requires a low forage level in order to achieve an outstanding animal growth. Under these conditions ruminal fermentation can be impaired by low ruminal pH reducing fiber digestion (Hoover, 1986). Adding fibrolytic enzymes to the diets can enhance ADF digestion (Beauchemin *et al.*, 1995) and energy availability, leading potentially to a higher animal performance in dairy cattle (Beauchemin *et al.*, 1999), beef cattle (Beauchemin *et al.*, 1995) and lambs (Titi, 2003). However, research conducted with fibrolytic enzymes in finishing programs of lambs is limited and dose levels of these additives are not well defined.

The objective was to evaluate the effect of four levels of Fibrozyme® added to the finishing diets of lambs on animal performance.

Materials and Methods

This research was conducted in the facilities of Facultad de Zootecnia y Ecología from Universidad Autónoma de Chihuahua, México. Thirty two male weaned lambs of commercial crosses with an average weight of 20.6 +/- 2.3 kg, and 90 days old were used. Prior to the experiment lambs were treated for internal and external parasites and vitamins A, D and E were injected, allowing for 15 d of adaptation period. Lambs were randomly assigned to four levels of Fibrozyme® (Alltech Inc., Nicholasville, KY): 0.0 (T-0.0), 0.025 (T-0.025), 0.05 (T-0.05) and 0.1 gr/kg of body weight (T-0.1), which were added to concentrate. Animals were allotted to individual pens and were fed ad libitum once daily at 0800 (Table 1). An initial finishing diet with a forage:concentrate ratio of 30:70 during the first 28 d of the experiment, and a second finishing diet from the 29 to 56 d with a forage:concentrate ratio of 20:80. Lambs were weighted every 14 d, and DMI was determined daily and individually.

Table 1. Ingredients and chemical composition of experimental diets.

Item	DM (%) (initial)	DM (%) (final)
Ingredients		
Alfalfa hay	19.9	20.0
Corn ground	41.9	58.5
Cottonseed meal	30.8	14.7
Molasses	3.58	3.53
Corn gluten	2.00	1.96
Mineral and vitamin premix	0.95	0.47
Calcium carbonate	0.51	0.33
Salt	0.45	0.44
Chemical composition		
DM	89.4	88.4
CP	21.4	16.0
NDF	3.58	6.18
ADF	1.21	2.09
ME (Mcal/kg DM)	2.50	2.92

Data from DMI, change of body weight, ADG and GE were analyzed using the PROC MIXED of SAS (SAS,2005) using the model: $Yij = \mu + Fi + Dj + Eij$; where: Yij = variable of response (Y1= DMI, Y2= change of body weight, Y3= ADG and Y4= GE), μ= general mean, Fi= Fibrolytic enzyme level, Dj= weigthing day effect, and Eij= random error, $Eij \sim IN(0, \sigma^2)$. Three lambs were discarded from the trial by health problems not related with the experiment protocol, therefore experimental treatments were: T-0.0 (n=7), T-0.025 (n=8), T-0.05 (n=7) and T-0.1 (n=7).

Results and Discussion

Dry matter intake was not improved by adding the fibrolytic enzyme under the levels sugested (P>0.05). The average DMI during the whole experiment was 1.76 kg/a/d (Table 2). Effect of fibrolytic enzymes on DMI is not clear. Flores (2004), observed similar results on DMI in dairy ewes (2.99 vs. 2.99) fed a 70:30 forage:concentrate diet added with 0 and 0.47 ml/kg of concentrate. Lack of effect of fibrolytic enzymes has been observed also in beef bulls (Villalobos *et al.*, 2007) and beef heifers (Titi and Tabbaa, 2004). However, Pinos-Rodríguez et al. (2002) found a higher DMI (1.35 vs 1.48 kgMS/a/d; P < 0.01) in sheep fed a diet added with Fibrozyme® (0 vs. 5 g/a/d). Also, Tous (2007) reported an increase in DMI (921 g/d vs 1139 g/d) in fattening lambs by adding the fibrolityc enzyme Biocellulase $^{A-20}$ (0.0 vs. 16.5 mg/kg as feed) and Titi, (2003), 839.9 vs. 888.8 g/a/d) when used Maxicel $^{200®}$(0.0 vs.150 g/Ton forage; P < 0.05).

Body weight change was not affected by experimental treatments (Table 2). Lambs achieved in 56 d of trial an average body weight of 40.7 kg. Similarly, ADG in lambs was not affected (P>0.05) by feeding Fibrozyme®, with an average of 0.31 kg/a/d. Probably, this in part is a reflect of the absence of an improvement in DMI by fed the fibrolytic enzyme. But, there is also controversy in regard with this, since Titi (2003) found a better growth response 0.22 vs 0.14 kg/a/d, using 150 g versus 0 g/Ton forage of Maxicel $^{200®}$, respectively.

As a result of no differences on DMI and ADG among treatments, GE was not improved by fed lambs with Fibrozyme®. The average of GE in the trial was 6.9.(Table 2). In the same manner as indicated above, there is discrepancy over the benefits of adding fibrolytic enzymes on GE. Titi (2003) indicated an improvement of 4.7 vs 7.59 on GE when fed lambs with Maxicel 200®, meanwhile Titi and Tabba (2004) reported an advantage of 11%. But Alvarez et al. (2009) did not find a benefit on GE (15.5, 13.9 and 14.4) by fed 0 g (control), 2 g/kgMS and 3 ml/kg MS of Fibrozyme® and Promote, respectively.

Implications

Adding Fibrozyme® to the finishing diets of lambs did not improve their performance. Probably, levels used of the enzyme as well as the forage source could be related to the lack of response. Undoubtely is a priority to enhance fiber digestion particularly under high concentrate diets to achieve better animal response and profitability of finishing programs of lambs.

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Table 2. Effect of Fibrozyme® on animal performance.

	TREATMENT			
TIME	T – 0.0	T – 0.025	T – 0.05	T – 0.1
DMI (kg/a/d)				
14 d	1.64 ± 0.11	1.62 ± 0.10	1.729 ± 0.11	1.65 ± 0.11
28 d	1.81 ± 0.11	1.77 ± 0.10	1.861 ± 0.11	1.80 ± 0.11
42 d	1.75 ± 0.11	1.68 ± 0.10	1.801 ± 0.11	1.83 ± 0.11
56 d	1.72 ± 0.11	1.69 ± 0.10	1.877 ± 0.11	1.90 ± 0.11
Mean (0-56 d)	1.73 ± 0.09	1.69 ± 0.09	1.817 ± 0.09	1.80 ± 0.09
ADG (kg)				
14 d	0.418 ± 0.36	0.384 ± 0.34	0.383 ± 0.36	0.362 ± 0.36
28 d	0.383 ± 0.36	0.272 ± 0.34	0.291 ± 0.36	0.321 ± 0.36
42 d	0.204 ± 0.36	0.273 ± 0.34	0.235 ± 0.36	0.235 ± 0.36
56 d	0.337 ± 0.36	0.280 ± 0.34	0.357 ± 0.36	0.291 ± 0.36
Mean (0-56 d)	0.336 ± 0.02	0.302 ± 0.02	0.316 ± 0.02	0.302 ± 0.02
Body weight change (kg)				
Initial weight	22.8 ± 1.48	22.7 ± 1.38	23.6 ± 1.48	23.5 ± 1.48
14 d	28.6 ± 1.48	28.1 ± 1.38	29.0 ± 1.48	28.5 ± 1.48
28 d	34.0 ± 1.48	31.9 ± 1.38	33.0 ± 1.48	33.0 ± 1.48
42 d	36.9 ± 1.48	35.7 ± 1.38	36.3 ± 1.48	36.3 ± 1.48
56 d	41.6 ± 1.48	39.6 ± 1.38	41.3 ± 1.48	40.4 ± 1.48
GE				
14 d	4.40 ± 2.11	4.32 ± 1.97	4.75 ± 2.11	4.73 ± 2.11
28 d	4.83 ± 2.11	7.54 ± 1.97	6.47 ± 2.11	5.75 ± 2.11
42 d	10.9 ± 2.11	6.50 ± 1.97	12.5 ± 2.11	13.0 ± 2.11
56 d	5.56 ± 2.11	7.20 ± 1.97	5.68 ± 2.11	6.83 ± 2.11
Mean (0-56 d)	6.41 ± 1.07	6.39 ± 1.00	7.34 ± 1.07	7.59 ± 1.07

T – 0.0 = Control; T – 0.025 = 0.025 g/kg of Fibrozyme®; T – 0.05 = 0.05 g/kg of Fibrozyme®; T- 0.1 = 0.1 g/kg of Fibrozyme®.

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EFFECT OF ZERANOL AND SEX CONDITION ON FINISHING HAIR LAMB PERFORMANCE

G. Villalobos[1], F. Núñez G[1], H. González-Rios[2], D. Domínguez[1], H.A. Castillo[1], J. Valles[1] and M. Lujàn[1].
[1]Universidad Autónoma de Chihuahua, Chihuahua, México.
[2]Centro de Investigación en Alimentación y Desarrollo A.C. Hermosillo, Sonora, México.

ABSTRACT: Hair sheep production in Mexico has become an activity of economic importance, and implants and castration are not of general use yet. In order to evaluate the effect of implanting zeranol and castration in the performance of finishing hair lambs 72 weaned lambs were used (21.4 kgï,±2.3), they were crosses of Blackbelly, Dorper, Kathadin y Pelibuey, and randomly assigned to treatments (n=18, 3 pens and 6 lambs per pen): intact lambs (IL); intact lambs and implanted with 12 mg zeranol (ILI); castrated lambs (CL); and castrated lambs implanted with 12 mg zeranol (CLI). Length of study was 70 d, lambs were fed *ad libitum* with a diet 80% concentrate (19% CP, 2.81 Mcal ME/ kg DM). Initial body weight (IBW) was recorded and every 14 d of the test (LW), average daily gain (ADG) per period, daily feed intake (FI), and feed efficiency (FE) were recorded. Data were adjusted with a factorial 2X2 arrangement, factors included were sex condition (SC; intact and castrated) and implanted (IMP) and non implanted (NIMP), using PROC MIXED (SAS). The model for ADG and LW included the fixed effect of the factors and their interaction, and sire breed (SB), ewe breed (EB), parturition type (PT) and as random effects pen and lamb, and IBW as covariable. For FI and FE, the model included only pen as random effect. Treatment mean were tested using LSMEANS/PDIFF. SEXC affected (P<0.05) LW, ADG, FI and FE in total length of the study. ADG was 32% higher and FE was 14% better in intact vs. castrated lambs. IMP also affected (P<0.05) lamb performance, IMP had 7% higher FI, 8% higher ADG than NIMP. Factor interaction was only observed between days 15 and 28 of the study, where IL, ILI and CLI showed higher ADG (P<0.05) than CL. It is concluded that lamb performance can be improved with zeranol implantation, and that intact implanted lambs perform better.

Key Words: finishing lambs, implants, zeranol, castration.

Introduction

In Mexico, lamb consumption is important, and because national production level is still low to satisfy the demand, around 40,000 tons are imported each year from different countries. (Sagarpa, 2006), which suggests the need for using technologies that allow us to increase lamb production.

Anabolic implants are used in different species and they improve production efficiency in 15 to 17%. Zeranol implants improved daily gain and feed efficiency in ram and wether lambs (Nold *et al.*, 1992).

Ram lambs are superior to wethers in lean carcass, growth rate, and feed efficiency (Seideman et al., 1982). Because some of the consumers complain about the flavor of intact lambs, castration is used in order to improve animal performance, in addition to modify some carcass and meat sensorial characteristics. In Mexico practices such as lamb implanting and castration are not yet common among commercial lamb producers, and there is little information on animal performance, meat and carcass characteristics of hair lambs.

The objective of this study was to evaluate the effect of castration and the use of zeranol on animal performance of hair lambs in feedlot.

Materials and Methods

The study was conducted at the Animal Science Department of the Universidad Autonoma de Chihuahua in Chihuahua, Mexico, 28º 35' north latitude and 106º 04' west longitude.

Animals, Facilities and Diet. There were used seventy two Pelibuey, Blackbelly, Dorper, Katadhin, and Suffolk terminal crossbred hair wether lambs with initial body weight of 21.4 ± 2.3 kg. Lambs were treated for internal and external parasites, vaccinated and vitamins A, D and E were applied, and individually ear tagged. All pens had free access to water. They had a diet adaptation period of 10 d. During experimental phase they were fed with the same diet of 20% forage and 80% concentrate (DM basis), it was formulated containing 19% CP, 2.81 Mcal ME/kg DM, and to get at least .220 kg of ADG (NRC, 1985; Table 1). Feed was served at 0800 and 1600 h.

Table 1. Experimental Diet composition (DM).

Ingredient	DM (%)
Alfalfa hay	20.0
Corn grain, ground	39.13
Corn dry distillery grain	18.00
Cotton seed meal (36% CP)	15.38
Soybean meal	5.00
Corn gluten meal (60% CP)	1.00
Mineral premix ®	0.50
Salt	0.50
Calcium carbonate	0.49

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Treatments. Lambs were randomly asigned to treatments (n=18, 3 pens and 6 lambs per pen): intact lambs (IL); intact lambs and implanted with 12 mg zeranol (ILI); castrated lambs (CL); and castrated lambs implanted with 12 mg zeranol (CLI). Castration and implanting were done during the adaptation period.

Body weight and average daily gain. Initial body weight (IBW) was recorded and every 14 d until 70 d of feeding (LW) in order to estimate ADG (kg) by period and total length of study. Lambs were weighed at 0800 h and after 16 h fasting.

Feed intake. Feed intake was recorded daily by pen, and average daily feed intake per period was calculated.

Feed efficiency. It was estimated by period for each treatment, as feed intake/ADG, and expressed as kg of feed per kg of gain.

Experimental design and statistical analysis. Data were adjusted with a factorial 2X2 arrangement, factors included were sex condition (SC; intact and castrated) and implanted (IMP) and non implanted (NIMP), using PROC MIXED (SAS). The model for ADG and LW included the fixed effect of the factors and their interaction, and sire breed (SB), ewe breed (EB), parturition type (PT) and as random effects pen and lamb, and IBW as covariable. For FI and FE, the model included only pen as random effect. Treatment mean were tested using LSMEANS/PDIFF. SEXC affected ($P<0.05$) LW, ADG, FI and FE in total length of the study.

Results

Initial body weight and LW for each period of the study are shown in Table 2. IBW was similar ($P>0.05$) among treatments, indicating that individuals were properly randomized and allotted to experimental treatment groups.

During initial 14 d of the study no effect ($P>0.05$) of the factors was observed on lamb LW. However, at 28, 42 and 56 d of the feeding period, an effect ($P<0.05$) of SC was observed, LW of intact lambs was higher compared to castrated lambs in 8%, 9% and 11% for 28, 42 and 56 d, respectively. Likewise, at 28 d, it was also observed an effect ($P<0.05$) of implanting, when implanted lambs increased their LW in 6.2% compared to non implanted lambs.

Table 3 shows the LS means of ADG for each period, as well as for the total length. ADG for periods 0-14 and 43-56 was affected by SC ($P<0.05$), being 25% and 32% higher in intact lambs compared to castrated animals, respectively. In the second period (15-28 d), an effect was found ($P<0.05$) for both factors and their interaction; being ADG of IL, ILI and CLI, similar among them (0.335, 0.369 and 0.368 kg., respectively) and different ($P<0.05$) to CL (0.237 kg.). ADG for periods 29-42 and 57-70, were similar ($P>0.05$) among treatments. For the total length of study an effect of SC and IMP, observing that intact lambs (IL + ILI) gained 19% more weigh ($P<0.05$) than castrated lambs (CL + CLI).

Implanted animals (ILI + CLI) gained 8% more weigh ($P<0.05$) than non implanted (IL + CL).

Average feed intake (Table 4) for each feeding period was similar among treatments ($P>0.05$), obtaining feed intake values close to 1.2 kg per animal per day in the initial phase of the study, and it increased up to 1.6 to 1.8 kg of feed per animal per day at the end of the test. For total length feed intake was affected ($P<0.05$) by IMP, implanted lambs consumed 7.24% more feed than non implanted lambs (1.55 vs. 1.45 kg).

Only in the period 43-56, and total length an effect of SC was observed ($P<0.05$) on feed efficiency (Table 5). Between days 43 and 56, intact lambs were 17.6% more efficient than castrated lambs (4.32 vs. 5.245 kg, respectively); and for total length intact lambs were 14% more efficient than those castrated (4.535 vs. 5.275 kg, respectively).

Discussion

Data of lamb performance obtained in this study are similar to those reported by Crouse *et al.*(1981), Nold *et al.*(1992) and Field et al. (1993), who also observed that intact lambs gained better with similar feed intake and better feed efficiency than wethers, their efficiency ranged from 8 to 25%. In contrast, Lirette *et al.* (1984) did not found differences in weight gain using Suffolk rams and wethers. Boggs et al. (1998), found that growth curve of castrated animals is less steep along the time than that of intact animals.

A reason for better animal performance in intact animals compared to those with gonads removed can be partially explained because the latter have no testicular androgenic hormones (testosterone and androsterone) that naturally promote animal growth, so that castrated lambs have a lower concentration of circulating growth hormone (Gerrard y Grant, 2003). In addition growth delay can be caused by the post traumatic damage, which can last a few days until full recovery (Lawrence y Fowler, 2002). This condition could happen to CL lambs during the first period of the study, who showed the lowest ADG; however, from the second period and on they had a light increase in ADG and according to the expected gain because of their diet.

Regarding the effect of Zeranol, our results are similar to those of reported in different studies (Hufstedler *et al*., 1996; Nold *et al.*, 1992), where the use of an hormonal implant in lambs has resulted in improved animal performance. Zeranol has a demonstrated estrogenic anabolic effect (Hofman, 1996), promoting efficiently muscle protein deposition, and reflected in improved animal growth (Gerrard y Grant, 2003). On the other hand, Olivares y Hallford (1990) using Devoulliet lambs observed an effect of zeranol improving ADG with an increased feed intake resulting in similar feed efficiency between implanted and non implanted.

Implications

Hair lamb performance under feedlot conditions can be improved by the implantation with 12 mg of zeranol; and the effect is enhanced in intact lambs.

Lamb castration decreased hair lamb performance, but this effect can be overcome by using zeranol implantation.

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Table 2. Average live weight (± se) of hair lambs per treatment.[1]

	Treatments			
Period (d)	IL	ILI	CL	CLI
Inicial	21.5±0.68[a]	21.6±0.64[a]	20.6±0.66[a]	20.6±0.70[a]
14 d	24.9±0.81[a]	25.5±0.79[a]	23.3±0.79[a]	24.0±0.81[a]
28 d[x,y]	29.6±0.91[b]	30.6±0.85[b]	26.6±0.85[a]	29.1±0.88[b]
42 d[x]	34.0±0.99[b]	35.6±0.93[b]	31.2±0.93[a]	32.7±0.99[a]
56 d[x]	39.57±0.62[b]	41.11±0.59[b]	35.08±0.61[a]	37.56±0.65[a]

[x], sex condition effect (P<0.05); [y] implanting effect (P<0.05)
[1]Adjusted with initial body weight as covariable.
[ab] Different literal within row are different (P<0.05)

Table 3. ADG (LSM± se) by treatments, period and total length (0-70 d).[1]

	Treatments			
Period	IL	ILI	CL	CLI
0-14 [x]	0.250±0.02[ab]	0.275±0.02[b]	0.186±0.01[a]	0.233±0.02[ab]
15-28 [x,y,z]	0.335±0.02[b]	0.369±0.02[b]	0.237±0.02[a]	0.368±0.02[b]
29-42	0.313±0.02[a]	0.353±0.02[a]	0.326±0.02[a]	0.288±0.02[a]
43-56 [x]	0.396±0.02[b]	0.392±0.02[b]	0.273±0.02[a]	0.322±0.02[a]
57-70	0.317±0.03[a]	0.292±0.04[a]	0.272±0.02[a]	0.266±0.03[a]
Total (0-70) [x,y]	0.328±0.01[b]	0.347±0.01[b]	0.268±0.01[a]	0.298±0.01[a]

[x] effect of sex condition (P<0.05); [y] and implanting effect (P<0.05); [z] interaction effect (P<0.05).
[1]Adjusted with initial body weight as covariable.
[ab] Different literal within row are different (P<0.05)

Table 4. Feed intake (LSM ± se) (kg) of hair lambs per treatment, period and total length. [1]

	Treatments			
Period (d)	IL	ILI	CL	CLI
0-14	1.24±0.08 [a]	1.26±0.08 [a]	1.11±0.08 [a]	1.33±0.08 [a]
15-28	1.37±0.12 [a]	1.57±0.12 [a]	1.29±0.12 [a]	1.60±0.12 [a]
29-42	1.45±0.07 [a]	1.52±0.07 [a]	1.35±0.07 [a]	1.50±0.07 [a]
43-56	1.66±0.20 [a]	1.71±0.10 [a]	1.52±0.11 [a]	1.59±0.93 [a]
57-70	1.76±0.06 [a]	1.67±0.23 [a]	1.64±0.13 [a]	1.80±0.13 [a]
Total (0-70) [y]	1.49±0.03 [a]	1.55±0.03 [b]	1.42±0.03 [a]	1.56±0.03 [b]

[y] Implanting effect (P<0.05).
[1]Adjusted with initial body weight as covariable.
[ab] Different literal within row are different (P<0.05).

Table 5. Feed efficiency (LSM ± se) (kg) in hair lambs per treatment, period and total length. [1]

	Treatments			
Period (d)	IL	ILI	CL	CLI
0-14	5.05±0.81 [a]	4.59±0.40 [a]	6.120±0.89 [a]	6.11±1.96 [a]
15-28	4.16±0.6 [a]	4.41±0.6 [a]	5.93±0.6 [a]	4.33±0.6 [a]
29-42	4.68±0.5 [a]	4.41±0.5 [a]	4.55±1.6 [a]	5.22±0.39 [a]
43-56 [x]	4.22±0.31[a]	4.42±0.63[a]	5.61±0.64[b]	4.88±0.57[a]
57-70	5.50±0.75 [a]	5.71±0.38 [a]	6.18±1.17 [a]	7.35±1.90 [a]
Total (0-70) [x]	4.61±0.13[a]	4.46±0.13[a]	5.33±0.29[b]	5.22±1.1[b]

[x] Sex condition effect (P<0.05).
[1]Adjusted with initial body weight as covariable.
[ab] Different literal within row are different (P<0.05)

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USE OF BROWN MIDRIB SORGHUM SILAGE IN CONDITIONING WEANED BEEF CALVES

D.Domínguez[*] , D.González, G.Villalobos, A. Anchondo, and J. Ortega.
Universidad Autónoma de Chihuahua, Chihuahua, México

ABSTRACT: Optimal use of traditional and good quality alternate forages is a necessity to alleviate the low weaning weight of calves produced in the extensive beef cattle system in northern Mexico. This study evaluated the effect of four treatments: corn silage (CS), conventional sorghum silage (CSS), brown midrib sorghum silage (BSS), and oat hay (OH) on the performance of weaned beef calves. Forty two male weaned calves Angus, Hereford and their crosses with an average body weight of 151.8 kg +/- 20.2 and approximately 210 days old were randomly assigned into 16 different groups of 3 individuals of similar initial body weight. Isoenergetic and isonitrogenous rations were formulated using forage at 75% DM and fed ad libitum during 54 d. Dry matter intake (DMI) was recorded daily, and the animals were weighted on days 13, 27, 41 and 54 on feed in order to estimate average daily gain (ADG) and gain efficiency (GE). Data were analyzed using PROC MIXED in SAS. Calves fed BSS had higher DMI ($P<0.05$) than those fed OH and CSS (4.8 vs. 4.3 and 4.2 kg, respectively). Feeding BSS enhanced ADG ($P<0.05$) vs. OH, CS, and CSS (0.99 vs. 0.47, 0.72, and 0.67 kg, respectively). Calves fed BSS and CS had higher GE ($P<0.05$) than calves fed OH (6.10 and 8.51 vs. 13.4). Final body weight was improved in calves fed BSS than in those fed OH, CS, and CSS (201.3 vs. 175.9, 190.3 and 185.9 kg, respectively). Calves fed BSS performed better than calves fed OH, CS and CSS.

Key Words: Brown midrib, sorghum silage, oat hay, corn silage, weaned beef calves

Introduction

The cow-calf system of northern Mexico is developed under extensive conditions with low availability and nutritive value of native forages during the extensive drought, driving to a low weaning weight of calves. Typically beef producers fed calves with diets based on low quality forages or oat hay harvested at hard grain stage, since concentrates are too expensive, affecting animal growth and profitability of the system (Domínguez et al., 2005). A potential alternative is the use of high quality and low cost production forages as brown midrib sorghum silage. The brown midrib mutation reduces the lignin concentration in sorghum by 21.5% and increases DMD by 21.3% and NDF digestion by 11.7%, leading potentially to a higher DMI and animal performance compared to conventional sorghum silage (Oliver et al., 2004).

The objective of this study was to evaluate the use of brown midrib sorghum silage on weaned beef calves performance.

Materials and Methods

This trial was conducted in Teseachi Experimental Ranch property of the University of Chihuahua, located in Namiquipa, Chihuahua, México. Forty two weaned and castrated calves Angus, Hereford and their crosses with an average body weight of 151.8 kg ± 20.2 and approximately 210 days old were randomly assigned into 16 different groups of 3 individuals of similar initial body weight, corresponding to four treatments: corn silage (CS, n=11), conventional sorghum silage (CSS, n=10), brown midrib sorghum silage (BSS, n=11), and oat hay (OH, n=10). The corn silage was hybrid 33G66 PIONEER; conventional sorghum silage was Silo Master ABT, brown midrib sorghum silage was sorghum bicolor BEEFBUILDER, and oat hay was Bachíniva genotype. Forage samples of OH, CS, CSS and BSS were analyzed for NDF, ADF, (Van Soest et a., 1991), and LDA (Goering and Van Soest, 1970) sequentially using the ANKOM^{200} fiber analyzer, and in vitro digestibility of DM and NDF was determined at 48 h using the in vitro system Daisy^{II200}. Calves were vaccinated, treated for internal and external parasites and vitamins A; D and E were applied prior to the beginning of the experiment, allowing for 10 d of adaptation period. Animals were fed ad libitum once daily at 0800 h during 54 d with isoenergetic and isonitrogenous diets containing from 71.0 to 76.7% forage on dry matter basis (Table 1). Dry matter intake was recorded daily by pen, and calves were weighted individually on days 13, 27, 41 and 54 d on feed in order to estimate ADG, body weight change (BWC) and GE. Data were analyzed using PROC MIXED of SAS 8.2 (SAS, 1999), using a complete random design with repeated measurements, adjusting a model that included as fixed effect forage source, and initial body weight as covariable.

Results and Discussion

Chemical composition of forage sources. The content of NDF and ADF was lower in BSS than CSS by 8.0 and 6.5%, respectively, but they were similar to CS.

Table.1 Composition of experimental diets

Item	OH	CS	CSS	BSS
	% of DM			
Forage	76.7	71.0	73.1	71.1
Ground corn	14.5	9.06	9.11	9.83
Soybean oil	2.01	1.12	2.00	2.15
Cottonseed meal	2.34	12.4	10.5	11.3
Corn gluten meal	2.26	3.48	2.97	3.21
Premix	0.83	1.15	0.98	1.06
Salt	0.34	.57	0.49	0.53
Calcium carbonate	1.01	1.22	0.79	0.86
	Chemical composition (% DM)			
NDF	50.9	60.7	63.5	58.4
ADF	29.0	31.7	35.6	33.3
Lignin	5.51	4.36	9.32	6.51
IVDMD	45.5	39.9	38.4	51.2
IVNDFD	34.7	32.6	29.2	40.9

OH= oat hay
CS= corn silage
CSS= conventional sorghum silage
BSS= brown midrib sorghum silage

Lignin content was reduced by 30.1% in BSS vs. CSS. The lower LDA content of BSS allowed for an increase on IVDMD (33.3%) and IVNDFD (40.0%) compared to CSS. Similar tendency of these results has been observed previously for brown midrib and conventional sorghum silage (Beck et al., 2007).

Animal performance. Calves fed BSS had an increase of 12.9% on DMI ($P<0.05$) than those fed OH and CSS (4.8 vs. 4.3 and 4.2 kg, respectively), but it was similar to CS (4.4 kg) during the whole experiment (Table 2). This response was consistently for the last 28 d of the experiment. Similar results have been reported in dairy cattle fed BSS vs. CSS, as DMI was improved by 8.7% (Oliver et al., 2004). Tjardes et al. (2000) also observed 13.9% higher DMI ($P<0.01$) in beef and dairy steers fed mutants of brown midrib corn silage versus the conventional corn silage (5.06 vs. 4.44 kg/a/d). The increase on DMI has been associated with the low lignin content that enhance NDF digestibility of the brown midrib mutants (Table 1) allowing potentially for an increase in rate of passage (Oba and Allen, 2000b). Feeding animals BSS enhanced ADG by 59.6% ($P<0.05$) vs. OH, CS, and CSS (0.99 vs. 0.47, 0.72, and 0.67 kg, respectively). This result is partially explained by the observed improvement on DMI, and probably by an increase on intake of digestible nutrients (Tine et al., 2001). Tjardes et al. (2000) did not find a benefit of fed brown midrib corn silage vs. its isogenic genotype on ADG, but it tended ($P<0.06$) to be increased (1.8 vs. 1.72 kg/a). Calves fed BSS and CS had higher GE ($P<0.05$) than calves fed OH (6.10 and 8.51 vs. 13.4). This response must be related to the higher ADG for calves fed BSS and CS. Probably the enhanced NDF of BSS could allow for an increase in energy availability, improving animal efficiency. Final body weight was improved by 9.4% in calves fed BSS than in those fed OH, CS, and CSS (201.3 vs. 175.9, 190.3 and 185.9 kg, respectively). Superior final weight must be the result of the increase on ADG during last 28 d of experiment.

Implications

Nutritional value of brown midrib sorghum silage was superior to conventional sorghum silage and oat hay. Calves fed brown midrib sorghum silage performed better than calves fed oat hay, conventional corn silage and conventional sorghum silage

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Table 2. Performance of weaned calves fed brown midrib sorghum silage.

Item		days of trial				Mean
		13	27	41	54	
DMI (kg)						
	OH	3.88[a]	4.21[ab]	4.49[a]	4.65[a]	4.30[c]
	CS	4.00[a]	4.43[ab]	4.55[a]	4.65[a]	4.40[bc]
	CSS	3.81[a]	4.11[a]	4.32[a]	4.76[a]	4.25[c]
	BSS	4.13[a]	4.64[b]	5.10[b]	5.37[b]	4.81[ab]
ADG (kg)						
	OH	0.40[a]	0.23[a]	0.77[a]	0.52[a]	0.47[c]
	CS	0.77[b]	0.68[bc]	0.75[a]	0.65[a]	0.72[b]
	CSS	0.54[ab]	0.53[b]	0.87[ab]	0.81[ab]	0.67[b]
	BSS	0.72[b]	0.94[c]	1.16[b]	1.07[b]	0.99[a]
BWC (kg)						
	OH	157.2[a]	159.1[a]	169.1[a]	175.9[a]	
	CS	161.4[a]	170.9[b]	181.3[b]	190.4[b]	
	CSS	159.5[a]	164.1[a]	174.7[a]	185.9[b]	
	BSS	161.2[a]	173.5[b]	188.5[c]	201.3[c]	
GE						
	OH	12.3[a]	22.0[c]	7.42[a]	12.9[b]	13.4[b]
	CS	5.69[a]	9.19[a]	8.63[a]	9.55[b]	8.51[a]
	CSS	11.8[a]	13.0[b]	6.28[a]	7.20[b]	9.28[ab]
	BSS	7.08[a]	5.35[a]	5.05[a]	5.79[a]	6.10[a]

OH= oat hay
CS= corn silage
CSS= conventional sorghum silage
BSS= brown midrib sorghum silage
Means in rows with different superscripts are different ($P < 0.05$)

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INFLUENCE OF RATIONS WITH MONENSIN AND TALLOW ON RUMINAL pH AND OXIDATION-REDUCTION POTENTIAL IN DRY DAIRY COWS

H. Castillo[1], M. Rivas[1], D. Dominguez[1], G. Villalobos[1] and J. Ortega[1].

ABSTRACT: The objective of this study was to evaluate the effect of a commercial ionophore feed additive (Rumensin200®) and tallow in rumen physical and chemical parameters relevant to methane production. For this purpose, four canulated Holstein dry cows (600 +/-20 kg live weight) housed in individual stalls were used in a 4X4 Latin square experimental design were allocated to 1 of 4 treatments: T1) total mixed ration (TMR) with a forage-to-concentrate ratio of 90:10, T2) TMR + 2 g of Rumensin200®, T3) TMR + 3.2% DM tallow and T4) TMR + Rumensin200® + 3.2% DM tallow. The cows were fed *ad libitum* twice a day at 700 and 1400 h. Oxidation-reduction potential (ORP) and pH were measured directly in the rumen at 0, 1, 2, 4, 8,12,18 and 24 hours after the first feeding. Statistical analysis of data was done using PROC ANOVA and GLM in SAS (SAS Inst. Inc., Cary NC) showed no significant differences in any of the measured variables among treatments. However, numerical values showed that the addition of Rumensin200® caused lower ORP values in T2 than in the other treatments (–261 vs. –253, –257 and –250 mV for T1, T3 and T4, respectively). Following a similar trend, pH was higher in T2 than in the other treatments (6.7 vs 6.6, 6.5 and 6,4 for T1, T4 and T3, respectively). These results suggest that Rumensin200® promotes more neutral pH conditions, probably by inhibiting the growth of H2-producing bacteria. The ORP stability observed among treatments suggests that in rations formulated for dry cows (high forage: concentrate ratio) neither Rumensin200® or tallow would have a positive effect in the cow's performance by reducing enteric methane production.

Key words: Rumensin, tallow, oxidation-reduction potential

Introduction

The ruminant contribution to global methane emissions has been perceived in recent years as a major environmental issue that influences current dairy production systems. On the other hand, methanogenesis in rumen represents an energy loss that approximates 6 to 8% of the total energy ingested by the ruminant. In dairy production systems methane synthesis reduction has been a subject of study in dairy and beef systems with the use of antimicrobial agents (Garcia-Lopez et al., 1996), feed additives (Benchaar et al., 2001) and microbial inoculants (Mutsvanga *et.al*. 1992), among others, fed directly as part of the ration. Eventhough most of these chemical compounds have a direct inhibiting effect upon the growth and physiological activity of specific members of the rumen microbial community, the modification of physical-chemical parameters of the ruminant forestomach such as pH and oxidation-reduction potential (ORP) also play an

important role in enteric methane production. The latter has been explained as a change in the molar proportion of the volatile fatty acids (VFA's) product of cellulose fermentation and in the modification of the H_2-accumulation in the ruminal gaseous phase.

Monensin, a ionophore commercially available as Rumensin200®, inhibits the growth of gram positive H_2-producing bacteria, therefore decreasing the concentration of H_2 gas required for CO_2 reduction into CH4. The effectiveness of Rumensin200® in *in vitro* and *in vivo* systems has been previously demonstrated (Odongo et.al.2007).

On the other hand, the use of ruminally-active tallow along with Rumensin200® has shown a decrease in the intake level in dairy goats which could limit the amount of cellulosic substrates available for methanogenesis.

This experiment was intented to characterized the changes in pH and ORP in the rumen due to the addition of monensin and tallow to a high forage:concentrate diet ratio typical of dairy cows during the dry period.

Materials and Methods

Animals, facilities and treatments: Four ruminally canulated Holstein dry cows (600 +/-20 kg live weight) were housed in individual stalls at the Universidad Autonoma de Chihuahua's Animal Science Department in Chihuahua, Mexico. The treatments were set up as follow: T1) total mixed ration (TMR) with a forage-to-concentrate ratio of 90:10, T2) TMR + 2 g of Rumensin200®, T3) TMR + 3.2% DM tallow and T4) TMR + Rumensin200® + 3.2% DM tallow.

Feeding and sampling: The cows were fed *ad libitum* twice a day at 0800 and 1400 h while pH and ORP were measured directly in the rumen at 0, 1, 2, 4, 8,12,18 and 24 hours after feeding, using a HANNA 9828 Multiparameter Sensor inserted directly in the ventral section and let to stabilize for 10 minutes before recording the readings.

Statistical analysis: Data was statistically analyzed as a 4X4 latin square using the ANOVA and GLM procedures of SAS (SAS Inst. Inc., Cary NC).

Results

Oxidation-Reduction Potential: There was not difference ($P > 0.05$). in ruminal ORP related to the addition of Rumensin200® or tallow. However, the interaction of Rumensin200® and tallow in T4 produced a wider ORP values, ranging from –234 to –294 mV. This was consistent with the fact that rations with either Rumensin200® or tallow exhibited the lowest ORP values. It was also observed that ruminal content was consistently more reducing (more negative values) right after the morning feeding (0800 and 0900 h) and started to gradually increase until it reached the initial value (Figure 1).

pH. The effect of treatment on rumen pH did not show a statistically significant difference ($P > 0.05$). However there was a consistent pH decrease after the first feeding of the day (0700 h). After the second feeding (1400 h) pH started to increase again towards initial values (hour 0). Direct observation of pH variability shows that addition of Rumensin200® with or without tallow produced a wider range: T2=6.14-7.11, T4=6.13-7.07 compared to T1=6.04-6.97, T3=6.07-7.01 (Figure 2).

Discussion

A decrease of H_2 concentration in the rumen gas phase

due to lower microbial activity and the hydrogenation of animal fats, such as tallow, can increase the concentration of CO_2 widening the range of the oxidation-reduction potential.

An increase in rumen pH on cows fed with rations that included Rumensin200® could be explained by the selective inhibition of H_2-producing bacteria, which is the main mode of action of this ionophore. A wider pH range in the ruminal content can select for a higher microbial diversity required for biological processes such as cellulose fermentation and methane production. These results are part of an ongoing research project that aims to explain the effects of monensin and tallow on the dynamics of methanogenic archaea and methane production.

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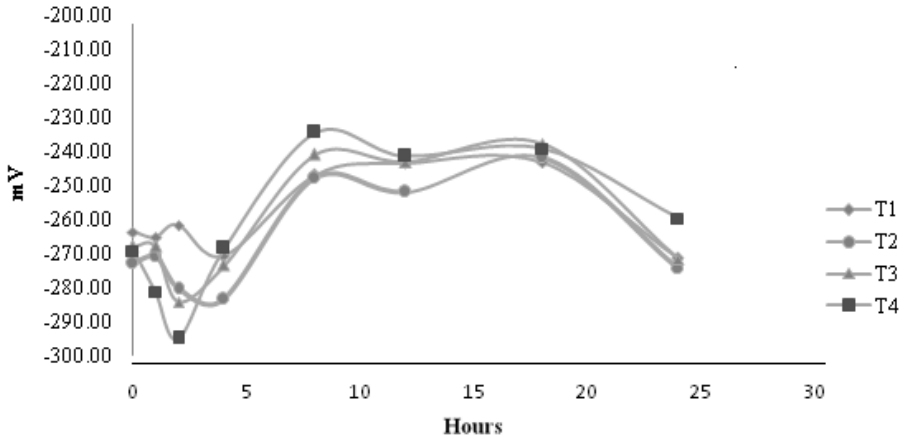

Figure 1. Dynamics of rumen oxidation-reduction potential in dairy cows fed a high forage:concentrate ratio diet with Rumensin200® and tallow. Values are means of n=8.

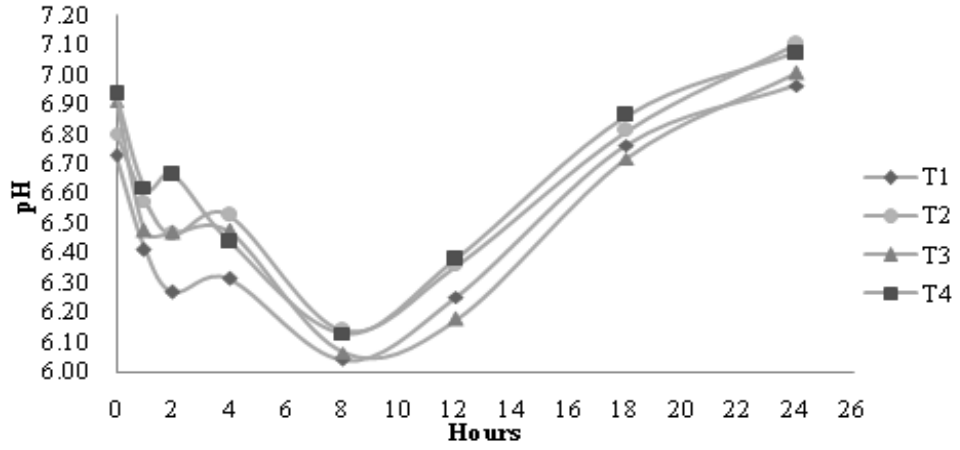

Figure 2. Dynamics of rumen pH in dairy cows fed a high forage:concentrate ratio diet with Rumensin200® and tallow. Values are means of n=8.

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RUMEN FERMENTATION AND PASSAGE RATE OF GROWING STEERS EXPOSED TO AN ENDOTOXIN

G. G. Gilliam, B. C. Graham, J. W. Waggoner, K. A. DeAtley, L. Chen, S. L. Lodge-Ivey, and C. A. Löest
New Mexico State University, Las Cruces, NM

ABSTRACT: Stressed calves during feedlot receiving may have altered gut motility and rumen function. The objective was to evaluate the effects of bacterial lipopolysaccharide (LPS) on rumen fermentation, passage rates, and diet digestibility in 20 ruminally cannulated steers (177 ± 4.2 kg BW). The experiment was a randomized block design, with 14-d adaptation to metabolism stalls and diet, and 6-d collection of feces. The diet (1.19 Mcal NE_g and 14.2% CP, DM basis) was fed twice daily at 1.5% of BW/d (DM basis). Treatments were a 2 × 2 factorial of LPS (0 vs ≥1.0 µg/kg BW; -LPS vs +LPS) and branched-chain AA (0 vs 35 g/d; -BCAA vs +BCAA). The LPS in 100 mL sterile saline was infused (1 mL/min via i.v. catheter) on d 15. The branched-chain AA in an essential AA solution were abomasally infused (900 mL/d) 3 times daily in equal portions beginning on d 7. Steers were offered 360 g of diet containing Cr-EDTA and 120 g of Yb-labeled diet 30 min before feeding on d 15. Fecal samples were retrieved from the rectum at 24, 48, 72, 96, and 120 h thereafter to calculate passage rate. Rumen fluid was collected at 0, 2, 4, 8, 12, and 24 h after LPS infusion on d 15. No LPS × BCAA × h, and no LPS × BCAA interactions ($P \geq 0.11$) were observed. Dietary OM and NDF intakes were lower ($P \leq 0.01$), total tract solid and liquid passage rates were slower ($P \leq 0.02$), and NDF digestibility tended to be greater ($P = 0.10$) for +LPS than -LPS steers. Rumen pH was lower for +LPS than -LPS steers at 4 and 8 h after LPS infusion (LPS × h; $P < 0.01$). Concentrations of acetate, propionate, butyrate, valerate, and total VFA were greater at 8 h, and isobutyrate and isovalerate concentrations were greater at 8, 12, and 24 h after LPS infusion for +LPS than -LPS steers (LPS × h; $P \leq 0.04$). Rumen NH_3 concentrations were greater for +LPS than -LPS steers at 24 h after infusion (LPS × h; $P < 0.01$). These results demonstrate that intravenous infusion of an endotoxin decreases total tract passage rate and alters rumen fermentation in growing steers.

Key Words: lipopolysaccharide, rumen fermentation, steer

INTRODUCTION

Morbidity due to exposure of newly received feedlot calves to infectious agents has a negative impact on performance (Waggoner et al., 2007). Among other factors (such as altered nutrient metabolism), low feed intake of newly received stressed calves likely contribute to decreased animal performance.

Cole and Hutcheson (1985) reported that limitations in feed intake could result in reduced ruminal fermentation capacity of pre-fasted feeder calves. Also, Galyean et al. (1981) reported that transportation stress affects rumen fermentation and alters rumen VFA concentrations, which could be a result of reduced rumen motility. Therefore, low feed intake and decreased animal performance may, in part, be affected by altered gastrointestinal digestibility, digesta passage rate, and rumen fermentation (Loerch and Fluharty, 1999).

In cattle, inflammation and stress in response to gram-negative bacterial pathogens can be induced experimentally with the infusion of purified bacterial lipopolysaccharide (**LPS**). Also, Waggoner et al. (2009) demonstrated that LPS infusion decreased passage of gastrointestinal solid and liquid contents, and probably altered rumen function. The objective was to study the effects of LPS exposure on rumen fermentation, passage rate, and diet digestibility of growing steers.

MATERIALS AND METHODS

Animals, Design, and Treatments

Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Twenty ruminally cannulated Angus steers (177 ± 4.2 kg initial BW) were housed in individual tie stalls of a metabolism barn with evaporative cooling. Steers were allowed free access to water and were limit-fed a wheat-based diet (Table 1) in two equal portions at 0700 and 1900. Daily DM offered was limited to 1.5% of BW to represent low intakes for newly received feedlot calves (NRC, 2000).

The experiment was a randomized block design. Steers were allowed 14 d to adapt to the facilities and diet before the beginning of a 6-d collection period. All steers received abomasal infusions (900 mL/d) of an essential AA solution in equal portions 3 times a day (0900, 1500, and 2100). The AA solution supplied 5 g L-Arg, 5 g L-His, 10 g L-Lys, 5 g L-Met, 5 g L-Phe, 5 g L-Thr, and 2.5 g L-Trp daily, and was infused via a flexible line (1/8 i.d.) that was placed through the rumen cannula and reticlo-omasal orifice. Jugular catheters (J-457A; Jorgenson Laboratories, Loveland, CO) were inserted for LPS infusion on d 14.

Treatments were arranged as a 2 × 2 factorial, and included 2 doses of LPS infusion (0 vs ≥1.0 µg/kg BW; **-LPS** vs **+LPS**) and 2 amounts of branched-chain AA supplementation (0 vs 35 g/d; **-BCAA** vs **+BCAA**). The LPS (*E. coli* 055:B5; Sigma Chem. Co., St. Louis, MO) was dissolved in 100 mL of sterile saline and infused (1 mL/min via i.v. catheter) at 3 h after feeding on d 15. The dose of LPS was initially 1.5 µg/kg BW (block 1), but after the death of a steer the LPS dose was lowered to 1.0 µg/kg BW (block 2). Sterile saline (100 mL) was infused into -LPS steers. The branched-chain AA (15 g Leu, 10 g Ile, and 10 g Val) were dissolved in the essential AA solution.

Table 1. Diet composition

Item	DM basis
Ingredient, %	
Wheat grain	30.0
Corn silage	21.3
Alfalfa hay	20.0
Soybean hulls	20.0
Molasses	4.0
Tallow	2.5
Minerals[1]	1.83
Urea	0.30
Vitamins[2]	0.04
Ruminsin-80[3]	0.02
Nutrient	
NE_g, Mcal/kg	1.19
CP, %	14.23
Ca, %	0.87
P, %	0.39

[1]Supplied (% of DM): limestone (0.50), dicalcium phosphate (0.50), sodium bicarbonate (0.50), salt (0.30), and trace minerals (0.03).
[2]Supplied 1,500 IU Vit A, and 100 IU Vit E per kg DM.
[3]Supplied 33 mg monensin per kg DM.

Collections

On d 15 at 0, 2, 4, 8, 12, and 24 h after LPS infusion, rumen fluid (100 mL) was collected via strainers (Precision Machine Co., Inc, Lincoln, NE) passed though the rumen cannula, and the pH was immediately recorded (Accumet AP72, Fisher Scientific, Pittsburgh, PA). An 8 mL sample of rumen fluid was added to plastic vials containing 2 mL of 25% (w/v) meta-phosphoric acid solution and frozen for later analysis.

Dietary samples were collected on d 15 through 19, and total feed refusals and total feces were collected on d 16 through 20 of the experiment. Steers were fitted with harnesses and collection bags for total fecal collection. Total feces was weighed, the weights were recorded, and 10% of the feces composited for each steer and frozen for later analysis. Passage rates were determine by feeding 360 g of diet containing Cr-EDTA and 120 g of Yb-labeled diet 30 min before the 0700 feeding on d 15. The Cr-EDTA and Yb-labeled diet was prepared as described by Waggoner et al. (2009). Samples of feces were retrieved directly from the rectum at 24, 48, 72, 96, and 120 h after feeding and frozen for later analysis.

Sample Analysis

Concentrations of NH_3 were determined in rumen fluid using the procedure of Broderick and Kang (1980) modified for a microplate reader (ELX 808 Ultra Microplate Reader, Bio-Tek Instruments Inc., Winooski, VT). Rumen VFA concentrations were analyzed using capillary gas chromatography (Varian 3400; Varian Inc., Walnut Creek, CA) in accordance to May and Galyean (1996).

Composite samples of diet, orts, and feces were dried at 55°C for 72 h in a forced-air oven (Model POM-326F, Blue M Electric Company, Blue Island, IL), allowed to air-equilibrate, weighed, and ground to pass a 2-mm screen (Model 4 Wiley mill, Thomas Scientific, Swedesboro, NJ). Ground samples were analyzed for DM (105°C for 24 h) in a convection oven (Precision Scientific, Chicago, IL), OM (500°C for 8 h) in a muffle furnace (Thermolyne Corp., Dubuque, IA), and NDF using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). Fecal grab samples were analyzed for Cr and Yb via inductively coupled plasma spectrometry (Optima 4300; Perkin Elmer, Wellesley, MA) as described by Waggoner et al. (2009). Solid (Yb) and liquid (Cr) passage rates (%/h) were determined from the slope of the natural log of Yb (48 to 120 h) and Cr (24 to 96 h) concentrations regressed against h.

Statistical Analysis

Data was analyzed statistically as a randomized block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The metabolism facility had only 12 tie-stalls, therefore steers were blocked by date of collection (8 steers in block 1, and 12 steers in block 2). For all dietary measures, the statistical model included effects of BCAA, LPS, and the interaction. For rumen pH, NH_3, and VFA concentration, the model included all combination of BCAA, LPS, and h using repeated measures subjected to the first order autoregressive covariance structure. Means were least squares, and significance was declared at $P < 0.05$.

RESULTS

No LPS × BCAA × h, and no LPS × BCAA interactions ($P \geq 0.11$) were observed. Also, effects of post-ruminal BCAA infusion on dietary measures and rumen fermentation were not significant ($P > 0.05$). Therefore, means for the effects of LPS on dietary intake, digestibility, and passage rates are presented in Table 2. Dietary OM and NDF intakes were lower ($P \leq 0.01$), and total tract solid and liquid passage rates were slower ($P \leq 0.02$) for +LPS compared with -LPS steers. Apparent digestibility of NDF tended to be greater ($P = 0.10$) for +LPS than -LPS steers.

Rumen pH (Figure 1) was lower for +LPS than -LPS steers at 4 and 8 h after LPS infusion (LPS × h; $P < 0.01$). Rumen NH_3 concentrations (Figure 2) were greater for +LPS than -LPS steers at 24 h after infusion (LPS × h; $P < 0.01$). Concentrations of acetate, propionate, butyrate, valerate, and total VFA were greater at 8 h, and isobutyrate and isovalerate concentrations were greater at 8, 12, and 24 h after LPS infusion for +LPS than -LPS steers (LPS × h; $P \leq 0.04$; Figure 2).

DISCUSSION

In this study, we limited the daily DM offered to 1.5% of BW so that intakes of the steers were representative of low intakes for newly received feedlot calves (NRC, 2000). Regardless of our attempts to limit intake differences among treatments, steers infused with LPS consumed less feed. This feed intake depression may be cause by various physiological factors associated with sickness and stress,

such as interactions of proinflammatory cytokines with the centers for intake regulation in the brain. Also, slower total tract solid and liquid passage rate for +LPS than -LPS steers indicates that intravenous infusions of LPS decreased gut motility, which also may have contributed to the decreases in feed intake. These responses are consistent with previous reports for endotoxin-challenged steers (Waggoner et al., 2009).

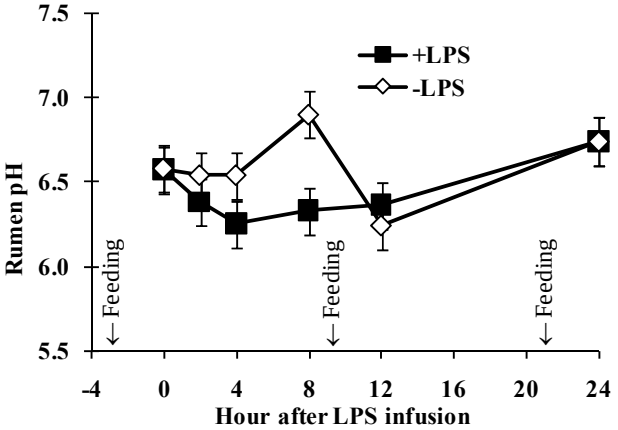

Figure 1. Effects of intravenous infusion (1 mL/min) of 100 mL sterile saline containing either 0 (-LPS) or ≥1.0 μg lipopolysaccharide per kg BW (+LPS) on rumen pH of growing steers. Effect of LPS × h ($P < 0.01$).

Slower passage rates generally result in greater retention of digesta in the gastrointestinal tract, and may explain a tendency for greater NDF digestibility by steers exposed to LPS. Also, rumen VFA concentrations were generally greater in +LPS steers, which is indicative of either increased ruminal carbohydrate fermentation, or decreased VFA absorption. Lower rumen pH in +LPS than –LPS steers like reflects greater VFA accumulation in the rumen. Galyean et al. (1981) also observed greater rumen VFA concentrations in steers exposed to transportation stress, and attributed the responses to decreased rumen motility and poor absorption of VFA. In our study, increases in rumen NH_3 suggest decreased N utilization by potentially compromised rumen microbial growth, or reduced NH_3 removal (absorption or passage) from the rumen. Cole and Hutcheson (1985) reported that pre-fasted feeder calves may have reduced ruminal fermentation capacity, but Fluharty et al. (1994) concluded that protozoa, but not cellulytic rumen bacteria, are reduced by fasting stress in calves. In conclusion, these findings demonstrate that intravenous infusion of an endotoxin decreases total tract passage rate and alters rumen fermentation in growing steers.

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Table 2. Effects of intravenous lipopolysaccharide (LPS) infusion on gastrointestinal tract apparent digestibility and passage rates of growing steers.

	Treatments[1]			
Item	-LPS	+LPS	SEM[2]	*P*-value[3]
Observations	10	8		
Intake, kg/d				
OM	2.36	1.88	1.68	0.01
NDF	1.04	0.83	0.077	0.01
Digestibility, %				
OM	79.3	81.3	1.15	0.19
NDF	70.6	74.9	2.00	0.10
Passage Rate, %/h				
Solid (Yb)	3.31	2.00	0.43	0.02
Liquid (Cr)	4.30	2.11	0.36	<0.01

[1]Intravenous infusion (1 mL/min) of 100 mL sterile saline containing either 0 (-LPS) or ≥1.0 μg LPS per kg BW (+LPS).
[2]Standard error of the mean (n = 8).
[3]Observed significance level for effect of LPS.

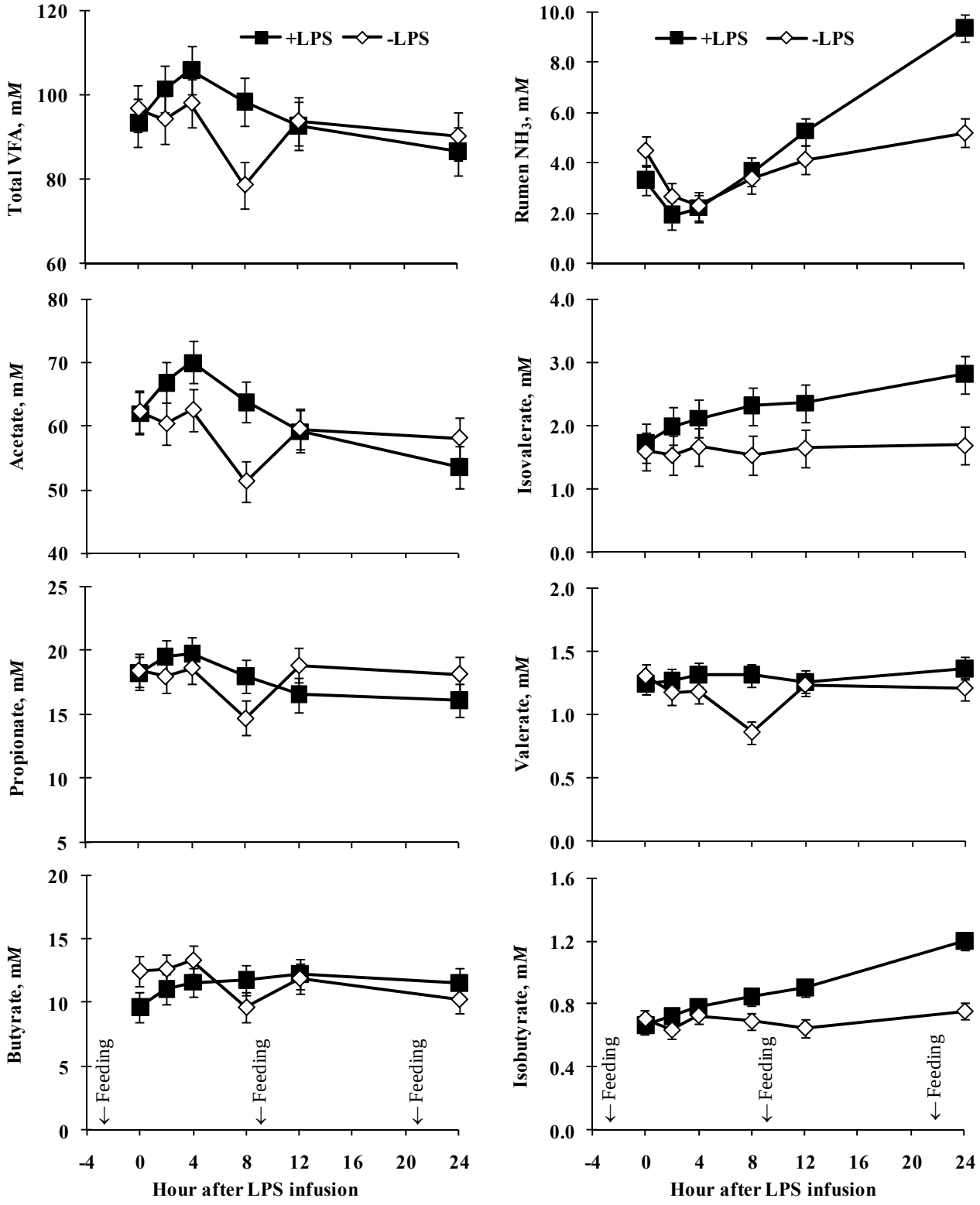

Figure 2. Effects of intravenous infusion (1 mL/min) of 100 mL sterile saline containing either 0 (-LPS) or ≥1.0 μg lipopolysaccharide per kg BW (+LPS) on rumen VFA and NH_3 concentrations in growing steers. Effect of LPS × h ($P <$ 0.01) for rumen NH_3; effects of LPS × h ($P \leq 0.04$) for acetate, propionate, butyrate, valerate, isobutyrate, isovalerate, and total VFA.

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EFFECT OF SUNFLOWER OIL SUPPLEMENTATION ON NUTRIENTS DIGESTIBILITY AND MILK FAT CONTENT OF CONJUGATED LINOLEIC ACID IN DAIRY CAMELS

S. N. AL-Dobaib
Qassim University, Buriedah-51452, P.O. Box 6622, Saudi Arabia

ABSTRACT: Three experiments were carried out to study the effect of sunflower oil (**SFO**) supplementation on nutrients digestibility (Exp.1), *in vitro* degradation kinetics of organic matter and fiber fractions (Exp. 2); and milk composition and fatty acids profile in milk fat of dairy camels (Exp.3). Chemical composition of the basal diet was 92.3%, 14.1%, 29.1%, 12.9% and 2.1%; of organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ether extract (EE), respectively. The SFO was added at the level of 0, 2 and 4% of DM for basal diet. Experimental diets were basal diet (**SF-0**) and basal diet with 2% SFO (**SF-2**) and basal diet with 4% SFO (**SF-4**). In digestibility trial (Exp.1), dry matter intake (DMI) and digestibility of NDF, ADF and N were significantly decreased ($P < 0.05$) by diet SF-4, but not with SF-2. Adding SFO at the level of 4% of DM negatively affected the ruminally degradable fraction and degradation rate of OM, NDF and ADF. Milk yield was significantly decreased ($P < 0.05$) when dairy camels were fed SF-4, however, no significant differences were detected on DMI and milk composition for either SF-2 or SF-4 (Exp.3). The principal aim of this study was to study the effect of different levels of SFO on the concentration of *cis*-9, *trans*-11 C18:2 in milk of dairy camels. The provision of FS-2 and SF-4 to dairy camel had no significant effect on the concentrations of capric acid ($C_{10:0}$) and layuric acid ($C_{12:0}$) of milk fat. Myristic ($C_{14:0}$) and palmitic acid ($C_{16:0}$) contents of milk fat of animals fed added-oil diets (i.e., SF-2 and SF-4) were decreased ($P < 0.05$) compared with SF-0. The concentrations of total short and medium chain FA (i.e. $C_{10:0}$ to $C_{16:0}$) were reduced by 38% and 48% with SF-2 and SF-4 than SF-0. A positive response was observed for *cis*-9, *trans*-11 conjugated linolenic acid (**CLA**) content in milk fat, which significantly increased ($P < 0.05$) by about 5 folds in animals fed SF-2 compared to SF-0. However, no significant difference was found between SF-0 and SF-4 in this respect. Total CLA isomers of milk fat were significantly ($P < 0.05$) higher in FS-2 than in other treatments, since the values were 0.94, 3.80 and 0.60 g/100 g fat for, SF-0, SF-2 and SF-4 respectively. Therefore, CLA content of dairy camels milk could be increased by the addition of SFO at the level of 2% of DM of the diet with no adherent effect on nutrients digestibility and daily milk production.

Key words*:* Dairy camel; Sunflower oil, CLA.

Introduction

Conjugated linoleic acid (**CLA**) represents a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds. The CLA are effective as anticarcinogenic, antidiabetic, and antilipogenic agents in the diet of laboratory animals (Pariza et al., 2001). Milk fat-derived *cis*-9, *trans*-11 C18:2 prevented growth of human mammary cancer cells more effectively than did synthetic *trans*-10, *cis*-12 C18:2 (O'Shea et al., 2000). Ruminant meat and milk are the predominant natural sources of the *cis*-9, *trans*-11 CLA, that accounts for nearly 90% of total CLA in milk fat from cows fed typical diets (Bauman et al., 1999). The *cis*-9, *trans*-11 CLA can be formed as a result of incomplete biohydrogenation of dietary fatty acids and by desaturase action on *trans*-11 C18:1 (another intermediate of biohydrogenation) in the rumen. It can also arise from isomerization via *cis*-12, *trans*-11 isomerase produced by rumen bacteria (Kepler and Tove, 1967). In the bovine mammary gland (Bauman et al., 1999) or human tissues (Pariza et al., 2001), *trans*-11 C18:1 can be a source for endogenous synthesis of *cis*-9, *trans*-11 CLA via Δ9-desaturase. The substantial variation in content of CLA in milk fat between herds suggests that diet has a major influence. Kelly et al., (1998) demonstrated that dietary supplementation of vegetable oils high in linoleic acid gave the greatest response, and there is a clear dose-dependent increase in milk fat content of CLA. Cruz-Hernandez et al. (2007) found that the addition of sunflower oil (i.e. 1.5%, 3.0% and 4.5% DM) to the diet in the percent of 0.5% of fish oil had no significant effect on milk production, and there was linear decreased in all short- and medium chain saturated fatty acids and a linear increase in total *trans*- 18-1 and total CLA.

Camel's milk is much more nutritious than that of cows. It is lower in fat and lactose and higher in potassium and its content of vitamin C is higher than that of cow's milk (Mehaia 1995; Farah 1993). Therefore, this study aimed to evaluate the effect of supplementing camels' diets with sunflower oil (**SFO**, that rich in unsaturated fatty acids) (**UFA**), on nutrients digestibility and fatty acid profile of milk in order to increase its nutritive quality for consumers.

Materials and Methods

Experimental location and diets. This study was carried out at the Agricultural Research Station, Qasssim University. Basal diet was formulated by Arabian Agriculture Services Company (ARASCO)-Riyadh, Saudi Arabia. Basal diet contained 92.3%, 14.1%, 29.1%, 12.9% and 2.1%; organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ether extract

(EE), respectively. Sunflower oil (SFO) was acquired from the local supermarket; it was added at levels of 0, 2 and 4% of DM for ground basal diet. Experimental diets were basal diet (SF-0) and basal diet with 2% SFO (SF-2) and basal diet with 4% SFO (SF-4).

Metabolism trial (Experiment 1). Three intact camel-claves (*Camelus dromedaries*) with body weight of 225 ± 11.3 kg, (mean ± SD) were used, diets and periods were randomly assigned for each animal in a 3 x 3 Latin square design. Each animal was kept in an individual metabolic cage having a tray for quantitative collection of feces and urine. The initial 21 days were used for adapting the animals, and the collection period of samples was carried out during the subsequent 7 days. During the collection period, daily feed intake and feces were recorded. Samples of feed and feces were collected every morning. One fifth of the weight of each daily feces voided by each animal was dried at 60˚C for 48 h, then was ground to pass through a 1 mm sieve and was preserved for chemical analysis.

***In vitro* rumen degradation of OM, NDF and ADF (Experiment 2).** By the end of the metabolism trial, animals were slaughtered to obtain rumen contents to carry out an *in vitro* experiment. Rumen content of each camel was squeezed through four layers of cheesecloth into pre-warmed flasks to separate the liquid from solid fractions. An automatic incubator (Daisy^{II} incubator; ANKOM Technology, NY, USA) with 4-glass bottles was used for the *in vitro* study. To begin the *in vitro* experiment, each glass was filled with 360 mL of rumen fluid and 1440 mL artificial saliva (Hungate 1966) and was kept in an incubator adjusted at 39 °C. Twenty-four bags were used for each treatment (pore size of 45 µm, Swiss Nylon Monofilament, Luzern- Switzerland). Six bags were incubated in each glass, then one bag was removed at intervals of 3, 6, 12, 24, 48 or 72h. Diets (i.e. SF-0, SF-2 and SF-4) were incubated with rumen fluid obtained from the animal fed the same diet. After the incubation, bags and residues were washed by running tap water until the water became clear, then they were squeezed gently. After washing, the bag contents were dried in an oven at 60 ºC for 48h and reweighed. Residuals of OM, NDF and ADF were determined in each bag. Degradability coefficients were calculated by fitting the data for OM, NDF and ADF disappearance to model of Ørskov and McDonald (1979). as following:

$$P = a + b\,(1\text{-}e^{-ct}),$$

where *P* is the cumulative amounts of OM, NDF and ADF degraded at time *t*, *a* is the readily degraded fraction, *b* is the fraction potentially degraded in the rumen, *c* is a rate constant of degradation of *b* and *t* is the incubation time in hours. Outflow rate was assumed to be 0.03 per h (AFRC 1993).

Feed intake and milk yield measurements (Experiment 3). Twelve multiparous she-camels (90±30 days postpartum; mean ± SD) were used to determine feed intake and milk yield as influenced by different levels of SFO supplementation. Each female was housed with its calve in an individual pen. The duration of milking trial was 8 wks. One day before the end of each week of lactation, calves were separated in the evening from their mothers and the mothers were machine milked for the remaining milk after calve suckling. Calves were kept near to their dames to stimulate milk secretion at both milking times (i.e. 06:00 and 18:00 h). On the last day of each week (milking and sampling day), does were totally machine milked in the morning and in the evening and milk yield was recorded and sampled for each female. Samples (100 mL from each) were divided into 2 equal subsamples, then 2 subsamples of 50 mL each (with and without potassium dichromate preservative) were stored for further analysis. Milk samples, with preservative, were kept at 4˚C for further analysis. Milk samples without preservative were kept frozen at -20˚C for fatty acids determination. Also, on the last day of each week of lactation trial diets were offered at 105% of the previous day's intake. The amount of feed offered and orts were recorded and sampled, then composited weekly according to treatment. Pooled samples of feed offered and orts were dried at 60˚C for 48 h to determine dry matter intake.

Laboratory analysis. Dry matter (DM), organic matter (OM) and ether extract (EE) and nitrogen (N) of feed and feces samples were determined according to AOAC (1990), while neutral detergent fiber (NDF), acid detergent fiber (ADF) were determined as described by Van Soest et al., (1991). Milk fat, N and Lactose contents of milk were determined using LactoStar (Funke-Gerber, Berlin-Germany).

Fatty acids analysis. Fat was extracted from 5 mL of milk using mixture of chloroform and methanol (2:1, v/v) as described by Folch et al., (1957). Fatty acids of milk fat were transmethylated using sodium methoxid. Fatty acids methyl ester (**FAME**) were separated on a Shimadzu 2010A gas chromatograph equipped with a FID detector, and a fused silica capillary column of 100 m × 0.25 mm i.d.; 0.2 µm phase film (SP 2380; Supelco, Inc., Bellefonte, PA). The split ratio in the injector port was 50:1 with a linear velocity of 25 cm/sec of He. Oven temperature was programmed to 60 °C for 5 min, then increased from 60°C to 170°C at 3 °C/min, held at 170 °C for 10 min, ramped to 230 °C at 5 °C/min. then hold for 20 min. Injector and detector temperatures were 250 °C. Fatty acids of milk fat were identified by comparison of their retention times with standard mixture of FAME (Cat.# O5632, Sigma-Germany & Cat.# 4-7123, Supelco, Bellefonte, PA-USA).

Statistical analysis. Data for metabolism trial were analyzed as a 3X3 Latin square design using StatView for Windows (SAS 1999) according to the following model

$$Y_{ijk} = \mu + T_i + P_j + A_k + e_{ijk}$$

where Y_{ijk} = observation, = overall mean, T_i = treatment (i = 1, 2 and 3), P_j = period (j = 1, 2 and 3), A_k = animal (k = 1, 2 and 3) and e_{ijk}= residual error. Results of feed intake, milk yield and composition and fatty acid content of milk fat, and results of the *in vitro* experiment were analyzed according to the following model

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} = observation, μ = overall mean, T_i = treatment (i = 1, 2 and 3), and e_{ij}= residual error. Tests of significance were performed using Tukey and Kramer test.

Results

Experimental diets contained similar concentrations of CP and metabolizabole energy (ME). The EE was increased in linear manner as a result of oil supplementation and the values were 2.1, 4.1 and 6.2% for SF-0, SF-2 and SF-4 respectively.

Table 1: Effect of different levels of sunflower oil on dry matter intake and nutrients digestibility in she-camel (Exp.1)

	Levels of sunflower			
	SF-0[1]	SF-2	SF-4	SEM[2]
Dry matter intake (kg)	4.9[a]	4.7[ab]	4.2[b]	0.17
Digestibility coefficient (%)				
DM	81.1[a]	76.7[a]	70.3[b]	1.62
NDF	64.6[ab]	66.5[a]	58.6[b]	2.51
ADF	57.9[a]	59.2[a]	49.2[b]	2.34
N	73.4[ab]	77.5[a]	70.9[b]	1.76

[1]Diet + 0% sunflower oil (SF-0), Diet + 2 % sunflower oil (SF-2), Diet + 4 % sunflower oil (SF-4).
[2] SEM= standard error of the means.
[a,b] Means in the same row with different letters in their superscripts differ significantly ($P < 0.05$).

In the digestibility trial (Exp.1), the addition of sunflower oil to the basal diet decreased dry matter intake for SF-2 ($P > 0.05$) and SF-4 ($P < 0.05$) than SF-0, and the reduction was 5% and 15% compared to SF-0 (Table 1). A non-significant negative effect was noticed for FS-2 on digestibility coefficient of DM. As for NDF and ADF the digestibility coefficient were not affected ($P > 0.05$) by the treatment compared to SF-0. On the contrary, SFO supplementation at the level of 4% (i.e. SF-4) significantly ($P < 0.05$) reduced the digestibility of DM, NDF and ADF than SF-0. Digested N was decreased ($P < 0.05$) for SF-4 compared to SF-2, while no significant differences were found between FS-2 and neither SF-4 or SF-0 (Table 1).

Table 2: *In vitro*, degradation kinetics of organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of diets supplemented with different levels of sunflower oil (Exp.2)

	Levels of sunflower oil			
	SF-0[1]	SF-2	SF-4	SEM
OM				
a[2]	26.4	25.9	24.6	1.65
b[2]	56.3[a]	54.3[ab]	49.3[b]	1.48
c[2]	0.108[ab]	0.119[a]	0.094[b]	0.007
NDF				
b	55.1[a]	54.3[ab]	47.6[b]	2.36
c	0.062	0.059	0.052	0.006
ADF				
b	43.3[a]	41.9[a]	32.1[b]	1.98
c	0.041[a]	0.039[a]	0.031[b]	0.002

[1]Basal diet + 0% sunflower oil (SF-0), basal diet + 2 % sunflower oil (SF-2), basal diet + 4 % sunflower oil (SF-4).
[2] *a*, *b* and *c* are constants predicted by the exponential equation as proposed by Ørskov and McDonald (1979).
[a,b] Means in the same row with different letters in their superscripts differ significantly ($P < 0.05$).

Degradation parameters of OM, NDF and ADF in tested diets were presented in Table 2. The rapidly degradable fraction (*a*) of OM was not affected by the SFO supplementation. Ruminally degraded fraction (*b*) was decreased ($P > 0.05$) for SF-2 and SF-4 ($P < 0.05$) compared with the basal diet. Degradation rate (*c*, % per h) of OM in SF-0 was increased ($P > 0.05$) by 10% compared to SF-0, however, addition of SFO at the level of 4% of DM significantly ($P < 0.05$) reduced the *c* fraction compared with control (Table 2). The *b* fraction of NDF was numerically decreased for SF-2, however, it was significantly ($P < 0.05$) lower at the level of 4% SFO than SF-0 (Table 2). A non-significant inhibitory effect after addition of SFO was noticed for *b* fraction and *c* of ADF when the SF-2 was incubated, and that effect was significant in SF-4.

Results of milk composition as affected by different levels of oil supplementation are presented in Table 3. Milk fat content was found to increase by 7% in SF-2 compared to basal diet (Table 3).

Table 3: Means of dry matter intake and milk yield and composition of dairy camels fed diets supplemented with different levels of sunflower oil (Exp.3)

	Levels of sunflower oil			
	SF-0[1]	SF-2	SF-4	SEM[2]
Chemical composition of milk				
Fat, %	1.98	2.12	2.01	0.09
Fat yield (g/d)	210	239	216	10.5
Protein, %	2.52	2.66	2.70	0.08
Lactose, %	4.65	4.92	5.04	0.12

[1]Diet + 0% sunflower oil (SF-0), Diet + 2 % sunflower oil (SF-2), Diet + 4 % sunflower oil (SF-4).
[2] SEM = standard error of the means.
Means in the same row with different letters in their superscripts differ significantly ($P < 0.05$).

Higher milk yield (data are not presented) and fat content for SF-2 than SF-0 and SF-4 leaded to a higher fat yield by about 14% in FS-2 than the control diet. Contents of milk protein and lactose remained unchanged for all SFO levels (Table 3). Fatty acid concentrations (g/100 g FA) of milk fat were presented in Table 4. The provision of FS-2 and SF-4 to dairy camel had no significant effect on the concentrations of capric acid ($C_{10:0}$) and layuric acid ($C_{12:0}$) of milk fat. Myristic ($C_{14:0}$) and palmitic acids ($C_{16:0}$) contents of milk fat for animals fed added-oil diets (i.e., SF-2 and SF-4) were decreased ($P < 0.05$) compared with SF-0 (Table 4). Total short and medium chains FA (i.e. $C_{10:0}$ to $C_{16:0}$) were reduced by 38% and 48% with SF-2 and SF-4 when compared with SF-0. An increasing response was observed in the concentration of C18:0 for SF-2 ($P > 0.05$) and SF-4 ($P < 0.05$) *vs* SF-0. Oleic acid was significantly increased ($P < 0.05$) and was relatively higher in response to level of SFO supplementation. The $C_{18:2}$ content in milk fat did not differ due to SFO supplementation (Table 4).

A positive response was observed for the *cis*-9, *trans*-11 conjugated linolenic acid (CLA) content in milk fat, which significantly increased ($P < 0.05$) by about 5 folds in fat for animals fed SF-2 compared to SF-0. However, no significant difference was found between SF-0 and SF-4. Similar response was found for *cis*-10, *trans*-12 CLA contents in milk fat of animals fed SF-2 and the increment was significantly higher than SF-0. Total CLA isomers of milk fat were significantly ($P < 0.05$) higher in FS-2 than other treatments, since the values were 0.94, 3.80 and 0.60 g/100 g fat for, SF-0, SF-2 and SF-4 respectively. The ratio between saturated fatty acids to unsaturated fatty acids (SFA/USFA) of milk fat was decreased, which reflect the significant reduction of SFA in fat as a result of added SFO to the diet**.** Meanwhile, the sum of USFA was increased significantly for SF-2 and SF-4 compared to SF-0, and the values were 40.3, 48.8 and 49.1 mg/ 100 g fatty acids of milk fat for SF-0, SF-2 and SF-4 respectively (Table 4).

Table 4: Fatty acid concentrations in milk fat of dairy camels fed diets supplemented with different levels of sunflower oil

	Levels of sunflower oil			
	SF-0[1]	SF-2	SF-4	S.E.M[3]
Fatty acid (FA, g/100g) of total methyl esters				
Capric (10:0)	0.21	0.23	0.19	0.03
Layuric (12:0)	0.22	0.21	0.21	0.02
Myristic (14:0)	9.5^{a}	6.3^{b}	6.1^{b}	0.18
Palmitic (16:0)	16.5^{a}	9.7^{b}	7.2^{c}	0.53
Stearic (18:0)	33.3^{b}	34.8^{ab}	37.3^{a}	0.92
Oleic (18:1)	36.1^{b}	38.2^{b}	43.7^{a}	1.52
Linoleic (18:2)	2.3	2.85	3.3	0.51
CLA $_{18:2\ cis\text{-}9,\ trans\text{-}11}$[2]	0.51^{b}	2.53^{a}	0.32^{b}	0.15
CLA $_{18:2\ cis\text{-}10,\ trans\text{-}12}$	0.43^{b}	1.27^{a}	0.28^{b}	0.10
Total CLA	0.94^{b}	3.80^{a}	0.60^{b}	0.20
Categories of fatty acids (g/ 100 g FA)				
Saturated FA (SFA)	59.7^{a}	51.2^{b}	51.0^{b}	1.41
Unsaturated FA (USFA)	40.3^{b}	48.8^{a}	49.1^{a}	1.44
SFA/USFA	1.48^{a}	1.05^{b}	1.03^{b}	0.08

[1]Basal diet + 0% sunflower oil (SF-0), Basal diet + 2 % sunflower oil (SF-2), Basal diet + 4 % sunflower oil (SF-4).
[2]CLA = Conjugated linoleic acid
[3] S.E.M. = standard error of the means.
[a,b,c] Means in the same row with different letters in their superscripts differ significant ($P < 0.05$).

Discussion

Diets were formulated on the basis of chemical analysis of initial samples of ingredients; consequently, we anticipated that keeping the forage to concentrate ratio and energy the same between diets. However, NDF actually fed throughout the experiment for SF-2 and SF-4 were grater by about 1.3 and 3.6 units than SF-0, respectively.

Low digestibility of DM in the SF-2 and SF-4 (Table 1) could partially explain the linear reduction of DM intake that might be due to decreasing turnover of the digesta to post-ruminal tract. Sekine et al. (2003) reported that the capacity of the rumen to accommodate the bulky food is one of limiting factors for DM intake. In contrary to these results, several researchers found no adverse effects of supplemental fat on DM intake of ewes (Zhang et al., 2007), dairy cows fed canola seed (Khorasani et al., 1991), sunflower seed (Markus et al., 1996) or flaxseed (Mustafa et al., 2003). However, feeding supplemental fat in the form of oilseeds is expected to have less detrimental effects on DMI than if similar amount was added as free oil (Kennely, 1996).

The high NDF content in SF-4 (about 12% higher than SF-0) and high oil supplementation con attribute to low digestibility of NDF and ADF; and subsequently DM digestibility. This finding is confirmed by the results of degradation kinetics of OM, NDF and ADF (Table 2). Veira et al., (2001) found that the ruminal DM and NDF degradability of alfalfa hay were reduced by over 20% when soybean oil added at the level of 3% to the ration of dairy cows.

The reduction of OM ruminally degraded was associated with a marked depression in NDF and ADF degraded in the *in vitro* study (Exp.2) for SF-4 and the values were lower than SF-0 by 14% and 26%, respectively. Results of the current study are in agreement with those of Ikwuegbu and Sutton (1982), they reported that the inclusion of free oil in the diet of sheep reduced digestion of energy and OM in the rumen in a linear manner with increasing oil level added. Reduction of ruminally degradable *b* fraction of OM, NDF and ADF with diet SF-4 could be due to the adversary effect of oil supplement on rumen fermentation and fiber digestion. Disruption of ruminal digestion by addition of fat to the diet has been documented (Palmquist and Jenkins 1980; Zinn 1989) and is more pronounced when polyunsaturated fatty acids (**PUFA**) are fed relative to saturated fatty acids (Ferlay et al., 1991).

As for the non-significant effect of SF-2 on the ruminal *b* fraction of OM, NDF and ADF; and total tract digestibility of nutrients, it could be due to low level of supplemental oil added and its effect on ruminal fermentation. These results are consistant with Dutta et al. (2008) who fund that diet supplemented with low level of palm oil (2.5% of DM) had no effect on digestibility of DM, OM and crude fiber, while, increasing supplemental oil to the diet had reduced those parameters.

Apparent digestibility of nitrogen was not affected at the low level of supplemental SFO (SF-2), however it was 5.5% higher than the SF-0 (Table 1). Animals fed diet with 4% SFO showed a significant ($P < 0.05$) reduction in N digestibility. The lower digested N found in the current study could be due to the negative effect of fat on activities of rumen microorganisms, and subsequently the constant digestion rate of ruminal undegraded N in the small intestine would lead to increasing fecal N and reducing the total tract digestibility of N.

Supplementing camel diets with SFO had no significant effect on milk fat contents (%), while; SF-2 had higher fat content by about 8% and 14% compared to SF-0, SF-4 respectively. Fat supplementation in animal diets affects milk fat percentage and composition by different mechanisms. First, fat feeding may have negative effects on rumen fiber digestion, thus decreasing acetic and butyric acid production thus affecting the *de novo* fat synthesis in mammary gland (Griinari et al., 1998). Second, when fat is included in the ration the uptake and direct incorporation of long–chain fatty acids into triglycerides by mammary gland are increased (Palmquist and Jenkins, 1980). Therefore, milk fat content will respond to the balance between a fatty acid uptake and secretion by the mammary gland, resulting in a decrease in *de novo* synthesis.

Milk protein concentration in dairy camels was found not to be affected by the level of supplemental oil. Whitlock et al. (2003) showed no difference in protein concentration or yield when cows were fed either conventional corn (CC) or high oil corn (HOC). This is in contrast to Weiss and Wyatt (2000), who observed a decreased in milk protein concentration when cows were fed HOC silage compared with CC silage.

Overall, treatments have a negative effect in milk fatty acids having 16 or fewer carbons (Table 4). When supplemental fats are fed, the relative concentration of short-chain and medium-chain fatty acids decreased and that of long chain fatty acids increased because of *de novo* fatty acids synthesis and esterification in mammary tissue is reduced (Palmquist and Jenkins, 1980). It is interesting to notice that the concentration of C16:0 in milk fat for camels fed SF-0 was found to be 16.5 g/100 g fatty acids. However, palmitic acids comprised about 31.1g/ 100g fatty acids of milk fat for cows with the range of 25.5-46.1 g/ 100g fatty acids of milk fat (Murphy et al., 2008; Cruz-Hernandez et al.,

2007; AbuGhazaleh, 2008). Ney (1991) reported that the reduce of medium-chain fatty acids may represent an improvement in the profile of milk fat fatty acids as these fatty acids have been reported to constitute the hypercholesterolemic portion of milk fat.

Increases in the concentration of milk stearic acid with the added-oil diet (Table 4) can be attributed to the complete ruminal biohydrogenation of some mono- and polyunsaturated fatty acids supplied by the SFO in the diet.

The main objective of this study was to examine the effect of different levels of SFO supplementation to camel diets on milk *cis-9, trans-11* CLA. In the current study, the concentrations of *cis-9, trans-11* CLA and total CLA were increased by about 5 and 4 fold for dairy camels fed SF-2 compared with that fed the control diet respectively. Previous work has suggested that the biohydrogenation sequence of linoleic acid can lead to an increase in CLA levels in milk fat (McGuire et al., 1996). As for the effect of SFO on concentrations of *cis-9, trans-11* CLA and total CLA in milk fat of camels fed SF-4, it was lower ($P > 0.05$) than SF-0 and SF-2 ($P < 0.05$). This finding is in agreement with that of Gervais et al., (2005) who found a significant reduction in *cis-9, trans-11* CLA content of milk fat when dairy cows received gradual levels of a rumen-inert conjugated linoleic acid supplementation. Also, Onetti et al., (2001) reported a decline in *cis-9, trans-11* CLA content of milk fat when tallow was increased from 2% to 4% of DM as a supplemental fat.

The content of PUFA of milk fat of camels fed the basal diet was 40.3g/ 100g of fatty acids; this value was relatively similar to those of Najdi camel (45.3%) as reported by Sawaya et al., (1984) and that of Abu-Lehia (1989) that was 43.1% of total fatty acids in camels milk fat.

Implications

It can be concluded that adding SFO at 4% of DM would negatively affect nutrients digestibility. However, CLA content of camel milk would be increased by the addition of SFO at the level of 2% of DM with no adherent effects on nutrients digestibility and daily milk production.

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